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Full Length Research Paper

Drought stress and its intensity, the factor of strategies selection for drought tolerance in *Haloxylon aphyllum*

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Osmotic parameters of *Haloxylon aphyllum* were studied after inducing dryness. Water relations parameters with improving resistance to dryness of this species through inducing severe dryness and clarification of physiologic mechanisms of this plant in response to a low water and dryness were among the objectives of this study. For this purpose, the method of pressure chamber was employed. By this method, the pressure-volume curve was drawn and the parameters of water relations of the plant were obtained from analyzing them. A relatively mild dryness was induced to plants through a lack of irrigation. After two weeks, Haloxylon water potential reached -16.5 bars. A severe dryness was also induced to that but after four weeks of no irrigation, it was reduced to -27.2 bars. Relatively mild and severe dryness were repeated for six and 11 periods respectively. In both series of experiment, the control water potential that were being watered every two days once, remained fixed at about -12.7 bars. Based on the results, although the relatively mild dryness increased the elasticity of plant textures, but it had not a meaningful impact on its osmotic potential. Although the use of a relatively severe dryness decreased both osmotic potential and osmotic adjustment, but at the same time, it increased the elasticity too.

Key words: Haloxylon, water relations, water stress, drought resistance, osmotic potential, elasticity.

INTRODUCTION

Plants use two mechanisms which are: 1) tolerating dryness and 2) escaping from it in confronting with dryness (Turner, 1979; May and Milthorpe, 1962). *Haloxylon aphyllum* is among species which with the help of endurance mechanism is able to spend dry periods. These mechanisms have been studied in some of the species like various kinds of pine (Emadian, 1988; Grime, 1979; Bilan et al., 1979:1978; Youngman, 1965).

Haloxylon species like many other species, by osmotic adjusting or increasing the elasticity of cellular wall in the condition of water tension maintain their turgescence better and consequently tolerate better drought periods (Emadian, 1988).

H. aphyllum like other multi-functional plants of haloxylon type is of great value and importance in protecting and supporting breakable ecosystems like deserts.

It is such that as a live windbreaker prevents soil

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erosion (Tokasi et al., 2007; Safarnejad, 2005; Jafari et al., 2004), and as a correcting element increases the organic materials of soil and in a long term improves the structure of soil (Jafari et al., 2004), and increases the plant enrichment of the area under coverage (Bakhshi and Biroudian, 2008). In addition for those who live in deserts, it is an important source for the provision of fuel and fodder for cattle (Tokasi et al., 2007). As it has a broad emission spectrum in different soils from the viewpoint of texture (Javanshir et al., 1996), it can be noticed much more.

However, unfortunately, despite the high importance of haloxylon in biologic stabilization of sand lands, most of the planted plants in desert regions are facing the problem of dryness. Based on the confirmation of authorities and those in charge of desertification projects, after transferring the plants of *H. aphyllum* planted in vase to a natural plantation bed and despite observing principles related with post-plantation stages, after sometimes, a high percentage of transferred plants were afflicted with dryness and they had to be replanted. Taking action to replant instead of dried plants imposes relatively extravagant costs to the executive system of natural resources. According to the existing documents in the File Keeping Department of Agriculture Ministry of Iran, in average in each period, more than 10% of the vases with *H. aphyllum* were fading and became dried in the fields, and required replanting. In order to help reduce mortality percentage of planted plants in main beds of plantation, it seemed that placing one-year plants of *H. aphyllum* which were exposed to periodic dryness and possibility of their compatibility with unfavorable condition resulting reduction of rainfall and periodic droughts could be tested as an appropriate approach.

The main goal in conducting this research was firstly the study the changes of water relations parameters in order to improve resistance to dryness of *H. aphyllum* through induction of periodic dryness and also clarification of physiological mechanisms of this species in response to water shortage and dryness.

MATERIALS AND METHODS

The identified seeds of *H. aphyllum* were planted in plastic vases and were taken into care for one year. Plastic vases with an approximate capacity of three liters were selected in order to pave way for a better and a greater growth of the plants roots. The soil consisted of wind sand, soil and leaf-soil in proportions of 2, 1 and 1 respectively. Supply of necessary nutrition for plants during the experiment was done by including leaf-soil in the mentioned combination. At the same time, in order to prevent unwanted accumulation of water in vases, some fine holes were made in their bottom. After one year, the plants were transferred to a greenhouse.

Upon completion of one-month period of plants compatibility in greenhouse, the treatments of dryness induction was applied. For this purpose, a sufficient number of good and healthy plants were selected for the experiment. Half of them were considered for induction of dryness and the rest were selected as the controls that were being watered every two days once. In this research, two series of experiments were conducted. The plants of the experiment of the first series received 6 periods of 7 to 14 days of dryness. During this period, the water potential (before sunrise) of their plant was measured every two days once by using the pressure chamber and according to Scholander method (Scholander et al., 1965). At this state, the water potential of haloxylon plants at the end of each period was reduced to -16.5 bars. The experiment second series plants, in addition to the mentioned dry periods, received five periods of dryness of 14 to 28 days too. In this series of tests, water potential of *H. aphyllum* plant was reduced at the end of each period to -27.2 bars. Induction of stress in mild (14-7 days) and severe (28-14 days) stresses were 6 and 6+5 (11) periods (replications) respectively. So every place (points) on the curve 3 is resultant 6 stress periods, and on the curve 4 is resultant 11 stress periods (repetitions).

It is worth mentioning that in order to measure the water potential of plants, separate plants were considered and in each measuring, 5 plants were cut and used. Plants were watered fully at the end of dryness period. Their before sunrise water potential in the day after irrigation was increased by -5.3 to -4.3 bars. Also, the control plants water potential was increased from -12.7 bars in both series of test to about -9.7 to -8.7 bars.

The impact of dryness induction on the elasticity and osmotic parameters of *H. aphyllum* plants became possible through an analysis of pressure-volume curves. These curves were prepared by using *Sholander method* (Figure 1).

The horizontal axis is the exit liquid volume (W_e) and its vertical axis is balancing pressure or $(\Psi_w)^1$. Point E, is the place of intersection of osmotic line with W_e axis showing the rate of water which is exited under the infinite pressure from plant known as simplistic water (W_s). Point B is the place of conjunction of osmotic line with the axis of $(\Psi_w)^{-1}$ showing that Ψ_s of plant is in the condition of full turgescence.

As Figure 1 shows each pressure-volume curve has two outstanding parts: 1) curve parts which encompasses about 5 to 8 points; 2) direct part which includes 8 to 11 points. The direct part of curve was used to estimate Ψ_w, Ψ_p, & Ψ_s. According to the recommendation of Cutler et al. (1979), this action was performed by drawing a regression curve on at least 7 to 8 points of the last spots of pressure-volume curves. The overall equation of the curve is as follows:

$$(\Psi_{st})^{-1} = (\Psi_{s0})^{-1} - m\sum W_i$$

In the relations, $(\Psi_{s0})^{-1}$ is the inverse of primary osmotic potential of plant at full turgescence condition; m is the slope of regression which is under the influence of plant size and osmotic feature of texture and rate of exchange of plant water, $(\Psi_{st})^{-1}$ is the opposite of Ψ_s for t times of a pair from the data of pressure-volume and $\sum W_i$ is its corresponding accumulated quantity of exited liquid. With this assumption that the mentioned relation to be fully true in the considered range, by placing each pair of P_i and ΣW_i, it is possible to get the Ψ_s. The continuation of this line crosses the vertical axis in point B which specifies the inverse of osmosis potential in full turgescence $(\Psi_{s0})^{-1}$ (Tyree and Jarvis, 1982). Subsequently, the turgor potential (Ψ_p) of the plant in each point of pressure-volume curve was obtained through difference of Ψ_w and Ψ_s related to the same point. On the other hand, the correlation line cut off the horizontal axis in point E, which determines the volume of exited liquid from plant in an infinite pressure $[(\Psi_{s0})^{-1} m^{-1}]$ (Figure 1). Active osmotic water of plant ($W\Psi_s$) was calculated in form of $W_s (W_0 - W_d)^{-1}$ and its inactive osmotic water in form of $1- W_s (W_0 - W_d)^{-1}$ (Cutler et al., 1979).

The existing data in the part of curvature of pressure-volume curve was used to estimate the average of the absolute value of elasticity ($\bar{\varepsilon}$) of *H. aphyllum* (Tyree and Jarvis, 1982). To achieve

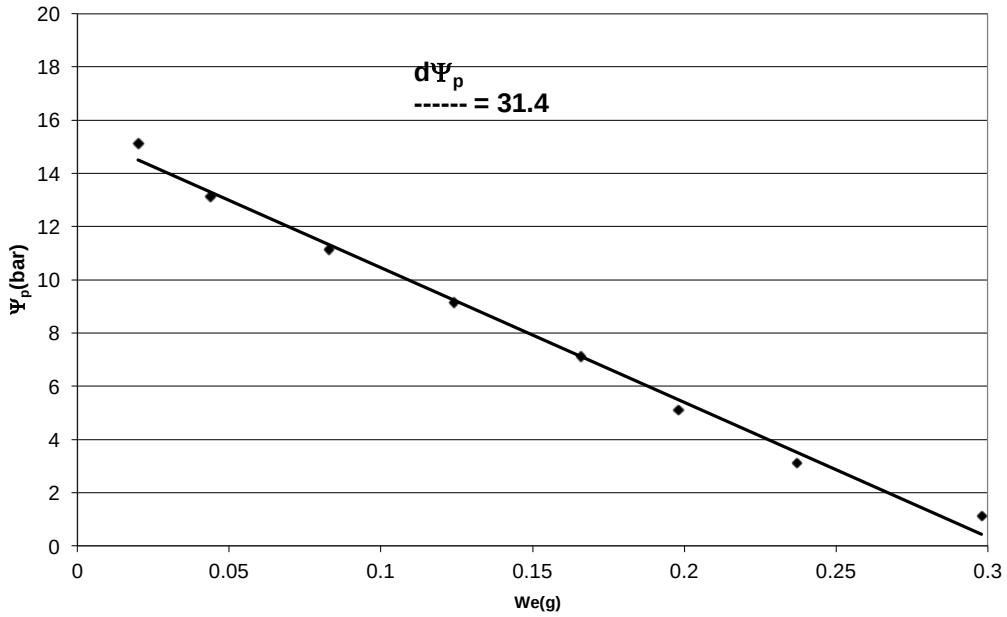

Figure 1. Pressure-volume curve of a plant of *Haloxylon aphyllum.*

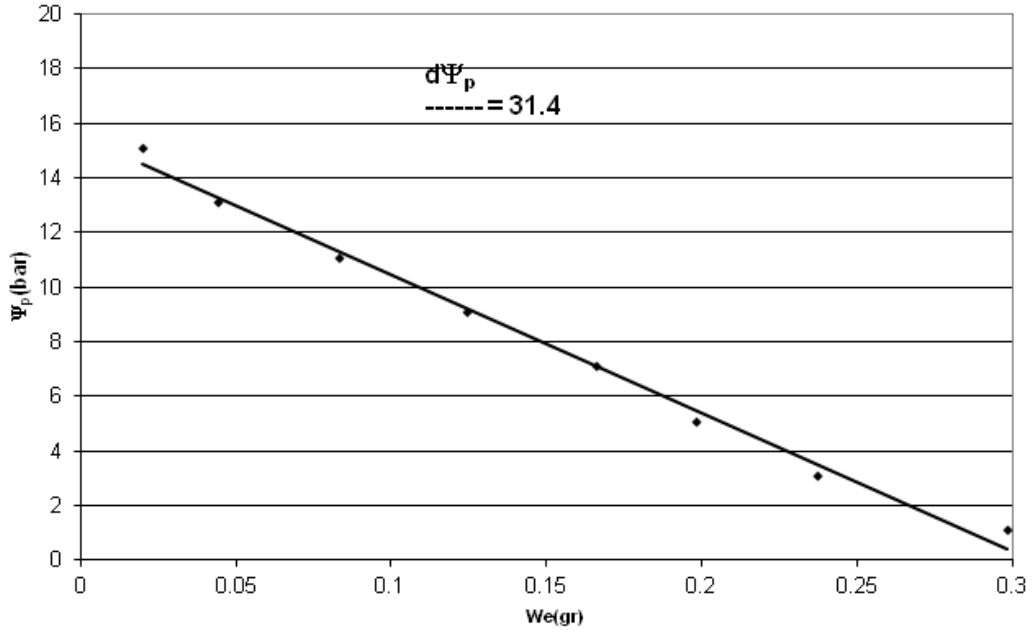

Figure 2. Calculating the rate of elasticity changes of cellular wall in *Haloxylon aphyllum* textures by using the curvature part of pressure-volume curve. Horizontal axis is exited volume (W_e), and vertical axis is swollen potential. In order to calculate the average of absolute value of the elasticity ($\bar{\varepsilon}$), the curve slope of $d\Psi_p\ (dW_e)^{-1}$ of plant was used.

this parameter, it was necessary that firstly Ψ_p of the plant in the part of curvature of the pressure-volume curve be calculated. This action was performed by using the osmotic line and method of estimation of osmotic, turgor and water potentials. Then the obtained turgor potentials [$(\Psi_p)s$] in the mentioned limit with the volume of corresponding condensed exited liquid was drawn in coordinates sheet and their regression equation was calculated (Figure 2). The ($\bar{\varepsilon}$) was also calculated from the product of the slope of line [$(d\Psi_p)\ (dW_e)^{-1}$] in Ws.

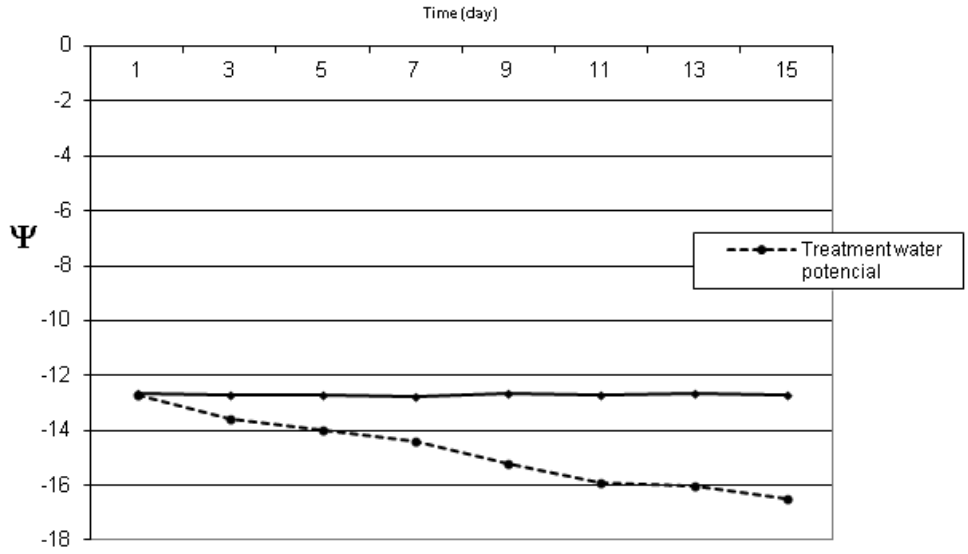

Figure 3. Relationship between plant water potential (Ψ_w) and time in *Haloxylon aphyllum* after watering. Pre-dawn plant water potential was measured every other day during two-week no-watering period.

Table 1. Comparing the result of water relations in *Haloxylon aphyllum* under mild dryness induction with control plants.

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_wTLP (Bar)	Ψ_sTLP (Bar)	ε (Bar)	WΨ_s (%)
STRESSED[1]	-2.50**	12.30**	-14.80	-20.90	-23.10	44.8**	14.00**
CONTROL[2]	-5.90	07.30	-13.20	-22.50	-21.40	30.50	28.80

[1]Seedlings, in addition to six early dry periods, five-day dry period 7 to 14 also received. The average water potential (before sunrise) at the end of their term limit had been reduced to -16.5 bars. Value of each parameter treated seedlings is related to seven seedlings. [2]Both seedlings were irrigated once a day. Amount of each parameter of control seedlings is related to five seedlings.

$\bar{\varepsilon} = W_s [(d\Psi_p)\ (dW_e)^{-1}]$

The used statistical method was t- student (Snedecor and Cochran, 1980).

RESULTS

Results of mild dryness induction

At the end of each period of mild dryness induction, water potential (Ψ_w) of *H. aphyllum* reduced about -16.5 bars; whereas Ψ_w of control remained at a higher range, that is in average -12.7 bars (Figure 3).

Parameters of active osmotic water (WΨ_s) and pressure potential at the condition of moisture full saturation (Ψ_{p0}) reduced and increased significantly and reached from 28.8 to 14% and was promoted from 7.3 to 12.3 bars, respectively. Their elasticity modulus ($\bar{\varepsilon}$) and water potential (Ψ_{w0}) had a highly significant increase (level 99%). Thus they accelerated from 30.5 to 44.8% and from -5.9 to -2.5 bars but the osmotic potential of tension treatments in full turgescence did not show a meaningful difference as compared with control (Table 1).

Results of severe dryness induction

After applying severe dryness induction, the water potential of *H. aphyllum* diminished in average up to about -27.2 bars, whereas the water potential of controls more or less remained fixed at the level of mild dryness induction (-12.7 bars) (Figure 4).

Water potential and pressure potential in fully saturation moisture condition increased significantly (P<1%), so that with the promotion to a higher level, it reached to the level of -2 and 18.8 bars respectively. At the same time, like the mild dryness tension, very meaningful increase of elasticity ($\bar{\varepsilon}$) and very meaningful reduction of osmotic active water (WΨ_s) were observed.

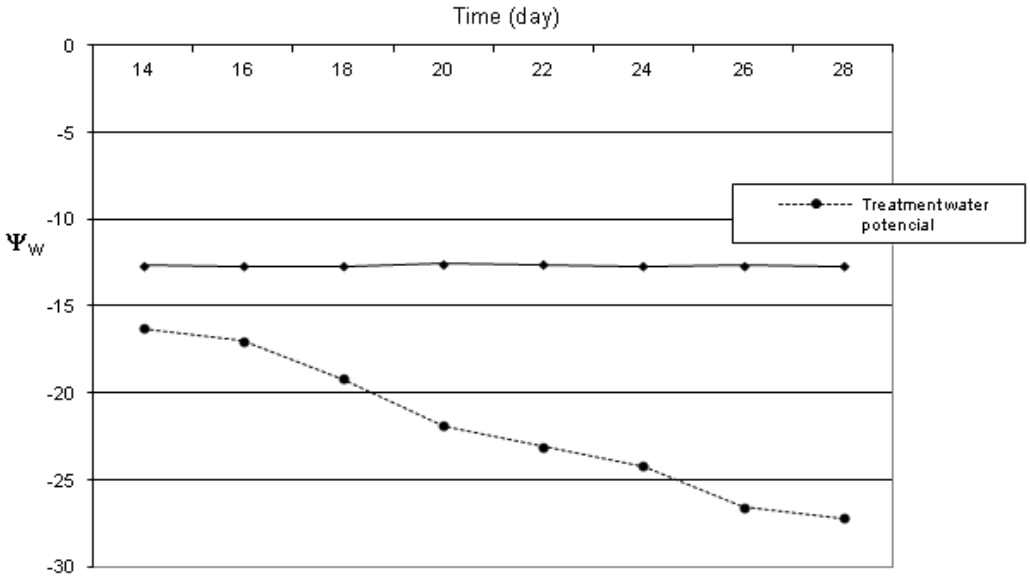

Figure 4. Relationship between plant water potential (Ψ_w) and time in *Haloxylon aphyllum* after watering. Pre-dawn plant water potential was measured every other day during a four-week period of no-watering.

However, concurrent with these changes, the osmotic potential in the condition of moisture full saturation (Ψ_{s0}) and dryness threshold (Ψ_{stlp}) reduced significantly (P<1%) and decreased from -13.5 to -20.8 bars and from -20.9 to -30.6 bars respectively.

DISCUSSION

Primarily it seemed that the reaction of parameters of water relations (like Ψ_{w0}, Ψ_{s0}, Ψ_{p0}, Ψ_{wtlp}, $\bar{\varepsilon}$) of *Haloxylon aphyllum* in mild dryness condition was in conflict with their corresponding parameters in the control treatment (Table 1), because measured absolute value of elasticity ($\bar{\varepsilon}$) of under the treatment plants was 44.8 bars that was more elastic than $\bar{\varepsilon}$ of control plants (30.5 bars) significantly. On the other, the measured osmotic active water ($W\Psi_s$) in treated and controlled plants was 14 and 28.8% respectively, and this difference was meaningful in the level of 99%. Possibly, the concept of this with regard to the relation of ($\bar{\varepsilon}$) is that the more elastic plant needs a less water to maintain its turgescence. In addition, in the condition of full turgescence, the water potential of plants which were given dryness was higher than water potential of control plants.

Plant physiologists believe that many plants of dry regions are able to maintain their turgescence through mechanism of elasticity and consequently increase resistance to dryness. This feature is true for *Pinus taeda* (Emadian and Newton, 1989), *Pseudosuga menziesii* (Joly and Zaerr, 1987), *Dubautia ciliotala* (Robichaux and Canfield, 1985), and *Juglans nigra* (Parker and Pallardy, 1985). On the other, though the elasticity of treated plants increased, but their osmotic potential was without any meaningful change. In other words, in condition of mild dryness induction, no meaningful difference was observed at any statistical level between Ψs_0 and Ψs_{tlp} in control and dryness tension treatments (Table 1). With regard to mentioned condition, it seems that in the mild dryness condition, *H. aphyllum* through increase of elasticity is able to maintain its turgescence and continues its growth. Apparently, this will be possible only in lieu of an exchange with reduction of osmotic adjustment.

Water relations parameters reaction in severe stress (28-14 days without irrigation) took place in continuing mild dryness condition, and osmotic adjustment was activated (Table 2) so that in addition to increase cell wall elasticity of shoot tissues (such as effect of periodic mild stress) osmotic potential of the tissue significantly decreased compared to control tissue, and continued the life of stressed-plants. After induction, severe periodic dryness on plants (*Haloxylon aphyllum*), the increase of water and turgor potentials (300 and 250%, respectively) and reduction of osmotic potential (more than 50%) in the condition of moisture saturation and at the dryness threshold was considerable.

The results showed that the induction of periodic mild dryness was not able to have an impact on the increase of resistance to dryness in *Haloxylon aphyllum*. It only changed the strategy of plant in confronting with stresses resulting from drought. Instead of that, periodic induction of severe dryness could make ideal changes in osmotic parameters, and not only caused the viable and

Table 2. Comparing the result of water relations in *Haloxylon aphyllum* under severe dryness induction with control plants.

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_wTLP (Bar)	Ψ_sTLP (Bar)	ε (Bar)	$W\Psi_s$ (%)
MDI	-2.50	12.30	-14.80	-20.90	-23.10	44.80	14.00
SDI	-2.00	18.80	-20.80	-20.30	-30.60	44.50	14.50

Table 3. Comparing the result of water relations in *Haloxylon aphyllum* under two water regimes: mild dryness induction and severe dryness induction.

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_wTLP (Bar)	Ψ_sTLP (Bar)	ε (Bar)	$W\Psi_s$ (%)
Stressed	-2.00**	18.80**	-20.80**	-20.30**	-30.60**	44.50**	14.50**
Control	-5.80	07.50	-13.50	-29.70	-20.90	31.80	26.50

[1]Seedlings, in addition to six early dry periods, five-day dry period 14 to 28 also received. The average water potential (before sunrise) at the end of their term limit had been reduced to -27.2 bars. Value of each parameter treated seedlings is related to seven seedlings. [2]Both seedlings were irrigated once a day. Amount of each parameter of control seedlings is related to five seedlings.

freshness of plant but also enabled plants to bear the severe condition of dryness and could maintain their water potential at a very high level. Comparing the parameters of water relations of *H. aphyllum* in mild dryness with severe dryness condition (Table 3) confirm the importance of the mentioned subject matter.

Mild dryness induction and severe dryness induction

Though under severe and longer dryness induction, cellular wall elasticity of *H. aphyllum* was maintained, however, osmotic adjustment became active for maintaining cellular turgescence. Therefore plants remained alive and continued their physiological and biochemical activities. Reduction of osmotic potential in the condition of saturation (by 6 bars) and reduce osmotic potential at threshold of wilt and dryness (by 7.5 bars) along with compatibility of plants to frequent dryness tension may enable them (compared to plants that were not exposed to any intensity of drought) tolerate drought periods resulting from factors affecting moisture shortage, and may be protected from the deleterious effects of drought periods in the same range of drought that seedlings similar to control wilt.

This idea can be put forth as the symbol of increase of resistance to dryness in *H. aphyllum* (which had received and accustomed to severe periodic dryness stresses) and to be used as a base for future research. Anyway, a decisive comment on this issue demands further studies and supplementary studies.

Conclusion

The results of this research showed that *H. aphyllum* tolerates dryness well. If dryness be mild and its period be short, this species will be able to maintain its turgescence and continue its life by increasing elasticity mechanism. Apparently, this action is possible only in lieu of an exchange with reduction of osmotic adjustment. On the other hand, if dryness be severe and its period be long, for keeping its turgescence, in addition to increase elasticity, it uses the mechanism of osmotic adjustment too. In each of the two mentioned condition, maintaining turgescence in dryness condition cause the continuation of physiologic and biochemical activities of this species and make its growth and viability possible despite excessive and long dryness. Based on the results of this research, the executive officials of forestry and production of plants departments recommend that if they use *Haloxylon aphyllum* for biologic stability of sand lands in arid and semi-arid zones, prior to transfer of plants to main field, they should place them under five to eight periods of dryness induction for at least three to four weeks.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Chemical profiles as chemotaxonomic tools for Loranthaceae in Nigeria

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The Loranthaceae species are widespread throughout most regions of the world, and are used for various medicinal and ethnopharmacological purposes. However, the species vary in their pharmacological activity, sometimes in correlation with the species from same ecological region or host plant, due to variation in the chemical profiles. This has led to great emphasis on caution in identification and collection for use. The wide array of secondary metabolites in Loranthaceae species are believed to be of chemotaxonomic importance. In this study, the leaves of seven Nigeria species from different ecological locations were screened for the profiles of their secondary metabolites with a view towards establishing chemotaxonomic significance. The results show the complete absence of alkaloid from all the species. Over 80% of the species tested positive for balsam, flavonoids and phenols, more than 70% tested positive for tannins, 60% for saponins and about 50% tested positive for glycosides and volatile oils. Resins, phlobatannin, terpenes, sterols and anthraquinones were present in less than 50% of the species. Some metabolites were completely absent in one or more species. The patterns displayed could be of chemotaxonomic importance for Loranthaceae in Nigeria.

Key words: Loranthaceae, chemotaxonomy, secondary metabolites, Nigeria.

INTRODUCTION

Mistletoes are widespread throughout Africa, North America, Asia, Europe, Australia and Malaesia, with the American mistletoe (*Phoradendron serotinum*) and the European mistletoe (*Viscum album*) particularly well known. Different species growing on different hosts may synthesize toxic compounds and protein such as lectins

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and alkaloids with varying pharmacological activities (Preston et al., 2010). Thus, both the mistletoe and its host have shared responsibility in determining the pharmacological activity of the species. The distributions of these compounds or metabolites in different parts of a plant also vary (Preston et al., 2010). These pharmacological effects are due to variation in the chemical profiles especially the profiles of secondary metabolites. Secondary metabolites are proteins, glycosides, phenolics, steroids, saponins, terpenes, alkaloids and other chemical substances. Takhtajan (1973) suggested that secondary metabolites are compounds that may have taxonomic relevance.

Eighty percent or more of the world's population is estimated to depend primarily on traditional medicine for the treatment of ailments (Cunningham, 1993), and as a matter of fact, the use of medicinal plants is the main means of treatment by traditional healers. Also, many useful compounds, which are today used for treatment of life threatening diseases, were isolated from medicinal plants e.g. Artemisinin from *Artemisia annua* L. and Vincristine from *Catharantus roseus* (L.) G. Don (Dana, 2012; Aslam et al., 2010). The Loranthaceae, a parasitic family with mistletoes members are often considered useful as medicinal plants. It has been documented that mistletoes have immeasurable medicinal and traditional uses (Burkil, 1995; Erturk et al., 2003). The biological activities of immunomodulatory and antitumor effect of some mistletoe may be attributed to the presence of metabolites like lectins, viscotoxins and alkaloids found in the parasites (Stirpe et al., 1982; Bussing et al., 1996; Fernandez et al., 1998; Stein et al., 1999; Mengs et al., 2002).

The wide array of secondary metabolites in Loranthaceae sp. is believed to be of chemotaxonomic importance. Chemotaxonomic studies of 12 Loranthaceae and Viscaceae species namely, *Viscum rotundifolium* L.f., *Viscum capensis* L.f., *Viscum combreticola* Engl., *Viscum obovatum* Harv., *Viscum obscurum* Thunb., *Viscum verrucosum* Harv., *Loranthus dregei* Eckl. & Zehy., *Loranthus minor* Sprague, *Loranthus oleifolius* (Wendl.) Cham. & Schltdl., *Loranthus rubromarginatus* Engl., *L. zeyheri* Harv. and *Loranthus* sp. were carried out in South Africa (Tilney and Lubke, 1974), and chlorogenic acid was found in all the species. Gedalovich-Shedletzky et al. (1989) analyzed and compared the chemical composition of viscin mucilage from three mistletoe species. Chemical analyses of different extracts from *Agelanthsu dodoneifolius* yielded components such as triterpenes, sterols, carotenoides, saponosides, anthracenosides, anthocyanosides and tannins (Traoré, 2000). However, chemotaxonomic information on the West African or Nigerian species is unavailable. To clarify the status of Loranthaceae in the region, a revision of the Nigerian species was carried out recently and about 15 species were documented for the region (Ibrahim and Ayodele, 2011). This study aimed to determine the profile of some basic secondary metabolites in the Nigerian species, which could be of chemotaxonomic significance.

MATERIALS AND METHODS

All reagents used were of analytical grade and were purchased from Zayo-Sigma Abuja, Nigeria. TLC plates used were also from the same source.

Plant collection and preparation

Twenty-seven specimens belonging to seven species were collected from the field through a field survey across host plant species and geographical location (Table 1). The specimens include *Agelanthus dodoneifolius* (4), *Globimetula braunnii* (4), *Phragmanthera capitata* (2), *Phragmanthera nigritana* (1), *Tapinanthus bagwensis* (4), *Tapinanthus cordifolius* (4) and *Tapinanthus globiferus* (8). Vouchers specimens were deposited at the University of Ibadan Herbarium (UIH)

The leaves of each specimen were air-dried for one week at ambient temperature, and then pulverized using a mortar and pestle. The powdered leaf samples were used for the phytochemical screening and thin layer chromatographic (TLC) profiling.

Phytochemical screening

The presence of basic secondary metabolites including saponins, alkaloids, tannins, flavonoids, sterols, phenols, glycosides, resins, balsam, volatile oil, phlobatannin, terpenes and anthraquinones were determined using standard methods (Evans, 2002; Sofowora, 1993; Brain and Turner, 1975; Segelman et al., 1971).

TLC profiling

Twenty-four specimens representing seven species were examined. The specimens are: *T. globiferus* (9), *T. bangwensis* (2), *T. cordifolius* (2), *P. capitata* (2), *P. nigritana* (1), *G. braunnii* (4) and *A. dodoneifolius* (4). A list of specimens and their corresponding numbers on the TLC plates are presented in Table 3.

Two grams of powdered leaf samples of each specimen were macerated in 20 ml of acetone for 24 h and filtered using filter papers. The extracts were spotted on three different pre-coated silica gel normal-phase TLC plates of dimension 12.5 by 8.5 cm. The dry spots were developed in a TLC tank of solvent system of ethylacetate : chloroform : methanol : water, in the ratio of 15:8:4:1. The developed spots were visualized by spraying the first plate with Vanillin in sulphuric acid reagent, the second plate with Gibbs reagent and the third plate with Dragendoff reagent for detection of terpenoids, phenolics and alkaloids, respectively. The retention factors (R_F values) were calculated for all the spots as distance moved by spot from the origin divided by distance moved by solvent front (Table 2).

RESULTS

Phytochemical screening

The result of the phytochemical screening for secondary

Table 1. Preliminary phytochemical screening of secondary metabolites from Loranthaceae species in Nigeria including taxa, hosts, localities, collection numbers and metabolites studied.

Taxa	Host	Locality/No.	Gly	Rsn	Blm	Fla	Tnn	Akd	V.oil	Ptn	Spn	Tep	Str	Phn	Atq
Agelanthus dodoneifolius	*Parkia biglobosa*	Jos 65		-	+	-	+	-		-	+	-	-	+	++
Agelanthus dodoneifolius	*Parkia biglobosa*	Suleija 77	+	-	-	+	+	-	+	-	-	-	+	+	
Agelanthus dodoneifolius	*Casuarina* sp.	Yola 119		+	+	+	+	-		-	+	-	+		-
Agelanthus dodoneifolius	*Vitellaria paradoxa*	Yola 118	-		+	+	+	-	-	-	+	+	-	+	
Globimetula braunnii	*Persea americana*	Calabar 90		-	+	-	+	-		-	+	-	-	+	-
Globimetula braunnii	*Cola* sp.	Calabar 92		-	+	-	+	-		-	++	-	-	+	+
Globimetula braunnii	*Cola* sp.	Ibadan 97	+	-	+	+	-	-	+	-	+	+	-		-
Globimetula braunnii	*Theobroma cacao*	Ibadan 102	-		+	+	-	-	-	-	+	+	-	-	
Phragmanthera capitata	*Persea americana*	Calabar 93		+	+	-	+	-		-	+	-	-	+	+
Phragmanthera capitata	*Persea americana*	Calabar 89		-	+	+	+	-	-	-	-	+	-		-
Phragmanthera nigritana	*Citrus* sp.	Chaza 78	-		+	+	+	-	-	-	+	+	-	+	
Tapinanthus bangwensis	*Newboldia leavis*	Ibadan 46		+	-	-	-	-		-	-	-	-		-
Tapinanthus bangwensis	*Citrus medica*	Ibadan 40	+	-	-	+	-	-	-	-	-	-	+	-	-
Tapinanthus bangwensis	*Cola acuminata*	Ibadan	-	-	+	+	-	-		-	+	+	+	+	-
Tapinanthus bangwensis	*Theobroma cacao*	Ibadan	-	-	+	+	-	-		-	+	-	-	+	-
Tapinanthus cordifolius	*Citrus auranthifolia*	Jos 63	+	+	+	+	+	-	+	-	-	-	-	+	
Tapinanthus cordifolius	*Cassia* sp.	Jos 86	-		+	+	+	-	-	-	+	+	-	+	
Tapinanthus.cordifolius	*Syzygium eucalyptoide*	Jos	+	+	+	+		-	+	-	+	+	+		-
Tapinanthus cordifolius	*Ficus sp.*	Jos	+	+		+		-	+	+	+	+	+		-
Tapinanthus globiferus	*Piliostigma thoninngii*	Kano 29		-	+	+	+	-		-	-	-	-	+	+
Tapinanthus globiferus	*Azadirachta indica*	Yola116		-	+	+	+	-		-	-	-	-	+	-
Tapinanthus globiferus	*Tectona grandis*	Kano 33		-	+	+	+	-		-	+	-	-	+	-
Tapinanthus globiferus	*Parinari curattelifolia*	Kano34	+	+	+	+	+	-	+	-	-	-	+	+	
Tapinanthus globiferus	*Zyzyphus* sp.	Yola 115		-	+	+	+	-		-	-	-	+		-
Tapinanthus globiferus	Unknown	Kano 27	+	-	+	+	+	-	+	-	-	-	-	+	
Tapinanthus globiferus	*Vitex doniana*	Suleija 73	-		+	+	+	-	-	-	+	+	-	+	
Tapinanthus globiferus	*Gmelina arborea*	Suleija 71		+	+	+	+	-		-	+	-	+		-

+ = Present; - = absent; ++ = abundant; Gly = glycoside; Rsn = resins; Blm = balsam; Fla = flavonids; Tnn = tannins; Akd = alkaloids; V.oil = volatile oil; Ptn = phlobatannin; Spn = saponin; Tep = terpenes; Str = sterols; Phn = phenols; Atq = anthraquinone.

metabolites of species of Loranthaceae on different hosts from different localities is presented in Table 1. Alkaloids were absent in all the species of Loranthaceae screened. Flavonoids were present in all except few specimens, *A. dodoneifolius* on *Parkia biglobosa* from Jos, the two specimens of *G. braunii* collected on an unidentified host from Calabar, *P. capitata* on *Persea americana* from Calabar and *Tapinanthus bangwensis* on *Citrus medica* from Ibadan (Table 1). Balsams occurred in all the specimens of the Loranthaceae species except few species like *A.*

Table 2. R_F values of phenolic spots from TLC profile of Loranthaceae species in Nigeria.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
														0.06	0.06		0.04	0.04			0.06			0.04
						0.07	0.10							0.09	0.10	0.07	0.09							
				0.12				0.14			0.14					0.14		0.13	0.14			0.12	0.12	0.13
						0.16						0.16	0.16				0.17					0.16	0.16	
				0.20			0.19																	
															0.23	0.25	0.25	0.25	0.25			0.25	0.23	0.26
	0.29	0.30		0.27	0.29	0.30	0.30	0.30	0.29	0.27	0.29	0.27	0.29	0.29	0.29	0.29	0.29	0.29	0.29		0.29	0.30		0.30
				0.32								0.32											0.32	
	0.36			0.37							0.35	0.36	0.36	0.36	0.35	0.35	0.35	0.35					0.37	0.37
							0.39					0.40			0.40					0.40		0.39		
											0.46							0.43	0.43			0.43		
$R_{F\ values}$ of spots	0.49		0.48	0.50		0.49	0.50	0.50	0.49			0.49	0.49			0.49		0.49	0.49	0.48	0.49	0.50	0.50	0.49
	0.53		0.53			0.52	0.53		0.52		0.53	0.52				0.53	0.52	0.53		0.53				
	0.56			0.55		0.56			0.55			0.56	0.56			0.56			0.55					
	0.59		0.59	0.58			0.59	0.59				0.59	0.58		0.58		0.59							
									0.63					0.63				0.63	0.63	0.63				
	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65				0.66	0.66			0.65	0.65				0.65			
												0.68						0.68	0.68	0.68		0.69	0.69	0.68
	0.73	0.73	0.73	0.72	0.72	0.72	0.72	0.72	0.72	0.71	0.71		0.71	0.71	0.71	0.71	0.71	0.72	0.71	0.71	0.71	0.73	073	
												0.78				0.76								0.75
								0.84									0.84	0.84	0.84	0.84		0.85	0.84	0.84
								0.91	0.91	0.91	0.92	0.91					0.91	0.91	0.91					
No. of spots	8	3	5	10	3	8	9	8	7	3	7	13	8	6	8	11	12	13	11	7	5	10	9	9

Key: See Table 3.

dodoneifolius on *P. biglobosa* from Suleija and *T. bangwensis* on *N. laevis* and *C. medica* (Table 1). Each of the four specimens of *T. bangwensis* on different host plants from Ibadan lacked tannins and also the two specimens of *G. braunii* from Ibadan lacked tannin (Table 1). Phenolics were also found to occur in most of the specimens screened except *G. braunii* on *Theobroma cacao* and *T. bangwensis* on *C. medica* (Table 1).

Figure 1 shows percentage response of the specimens to the metabolites screened. From this study, none of the metabolites occurred in all specimens or even all species (Figures 1 and 2). Figure 2 present the percentage of species responding to metabolites in each location while Figure 3 shows the percentage response to metabolites by the species. Generally, about 90% of the species tested positive for balsam and phenols, while 76% tested positive for tannins, 63% for saponins and less than 5% for phlobatannin (Figure 1). All the samples of *G. braunnii* tested positive to balsam and saponins, all *P. capitata* tested positive to balsams, flavonoids and tannins, *P. nigritana* tested positive to balsams, flavonoids, tannins, saponins, terpenes and phenols, while samples of *T. bangwensis* varied in their chemical profiles with no consistent positive indication for a particular metabolite. However, over 75% tested positive to balsams, flavonoids, and phenols, while about 75% also tested positive to glycosides, resins, balsams, volatile oils, saponins and terpenes. All samples of *T. globiferus* tested positive to balsam, flavonoids and tannins and 75% was positive to phenols.

TLC profiling

The TLC profiling of the specimens of

Table 3. List of specimens, hosts and their corresponding extract spot number on the TLC plates {(Figures 4-6) and Table 2}.

Specimen number on TLC plate	Name of Parasites	Name of Host	Host Family	Locality of collection/collection no.
1	*Tapinanthus globiferus*	*Piliostigma thoninngii*	Fabaceae-ceasalpinioideae	Kano 29
2	*T. globiferus*	*Azadirachta indica*	Meliaceae	Yola116
3	*T. globiferus*	*Tectona grandis*	Verbanaceae	Kano 33
4	*T. globiferus*	*Parinari curattelifolia*	Chrysobalanaceae	Kano34
5	*T. globiferus*	*Zyzyphus sp*	Rhamnaceae	Yola 115
6	*T. globiferus*	*Terminalia avicenoides*	Combretaceae	Kano 35
7	*T. globiferus*	-	-	Kano 27
8	*T. globiferus*	*Vitex doniana*	Verbanaceae	Suleija 73
9	*T. globiferus*	*Gmelina arborea*	,,	Suleija 71
10	*Tapinanthus bangwensis*	*Newboldia leavis*	Bignoniaceae	Ibadan 46
11	*T. bangwensis*	*Citrus medica*	Rutaceae	Ibadan 40
12	*Tapinanthus cordifolius*	*Citrus auranthifolia*	,,	Jos 63
13	*T. cordifolius*	*Cassia* sp.	Fabaceae-ceasalpinioideae	Jos 86
14	*Phragmanthera capitata*	*Persea americana*	Lauraceae	Calabar 93
15	*P. capitata*	*Persea americana*	,,	Calabar 89
16	*Phragmanthera nigritana*	*Citrus* sp.	Rutaceae	Chaza, Suleija 78
17	*Globimetula braunii*	*Persea americana*	Lauraceae	Calabar 90
18	*G. braunnii*	*Cola* sp.	Sterculiaceae	Calabar 92
19	*Globimetula braunnii*	*Cola* sp.	,,	Ibadan 97
20	*G. braunnii*	*Theobroma cacao*	,,	Ibadan 102
21	*Agelanthus dodoneifolius*	*Parkia biglobosa*	Fabaceae-mimosoideae	Jos 65
22	*A. dodoneifolius*	*Parkia biglobosa*	,,	Suleija 77
23	*A. dodoneifolius*	*Casuarina* sp.	Casuarinaceae	Yola 119
24	*A. dodoneifolius*	*Butryospermum parkii*	Sapotaceae	Yola 118

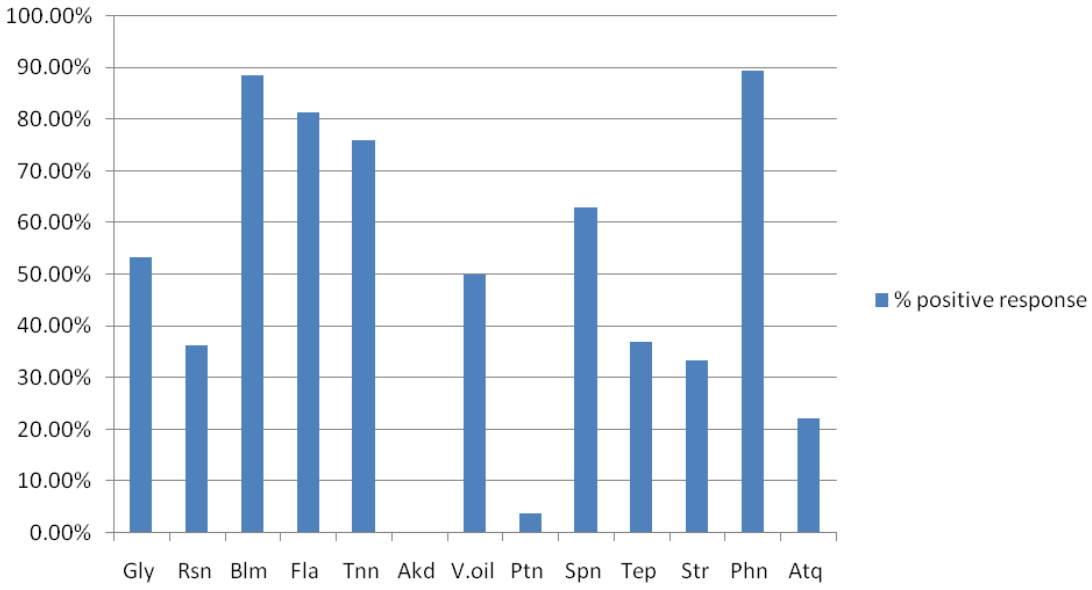

Figure 1. Percentage response of the total specimens of Loranthaceae species in Nigeria to presence of secondary metabolites. Gly = Glycoside; Rsn = Resins; Blm = Balsam; Fla = Flavonids; Tnn = Tannins; Akd = Alkaloids; V.oil = Volatile oil; Ptn = Phlobatannin; Spn = Saponin; Tep = Terpenes; Str = Sterols; Phn = Phenols; Atq = Anthraquinone

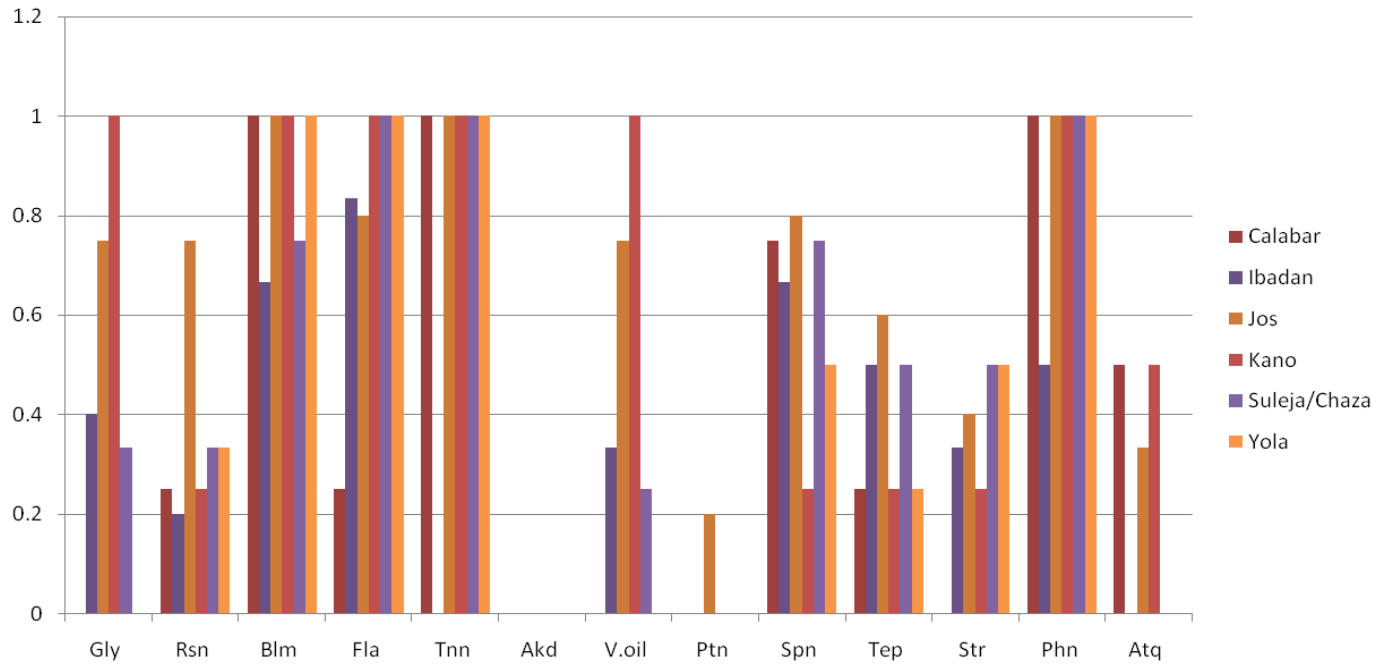

Figure 2. Percentage of species of Loranthaceae in Nigeria responding to secondary metabolites by location. Gly = Glycoside; Rsn = resins; Blm = balsam; Fla = flavonids; Tnn = tannins; Akd = alkaloids; V.oil = volatile oil; Ptn = phlobatannin; Spn = saponin; Tep = terpenes; Str = sterols; Phn = phenols; Atq = anthraquinone.

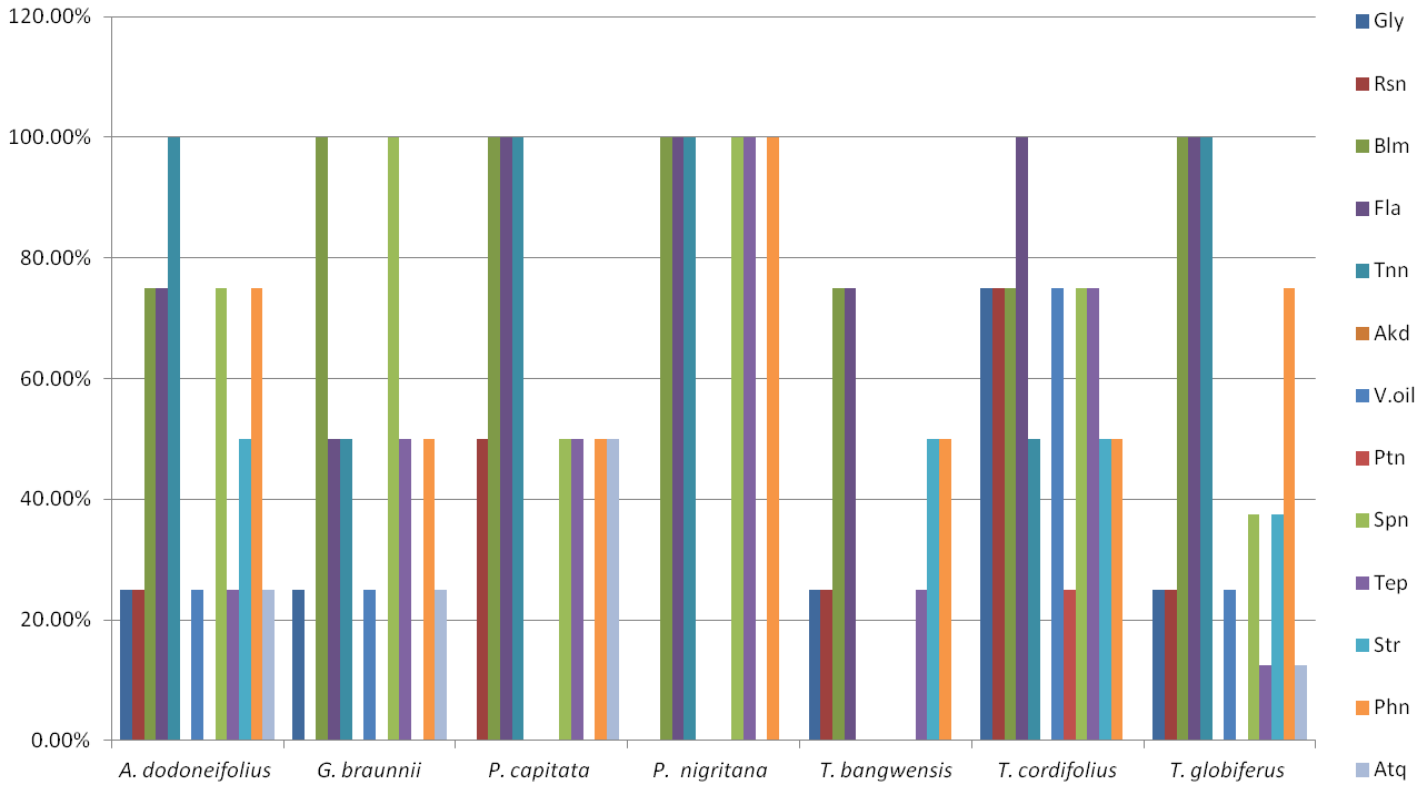

Figure 3. Percentage response of Loranthaceae species to secondary metabolites by species. Gly = Glycoside; Rsn = resins; Blm = balsam; Fla = flavonids; Tnn = tannins; Akd = alkaloids; V.oil = volatile oil; Ptn = phlobatannin; Spn = saponin; Tep = terpenes; Str = sterols; Phn = phenols; Atq = anthraquinone.

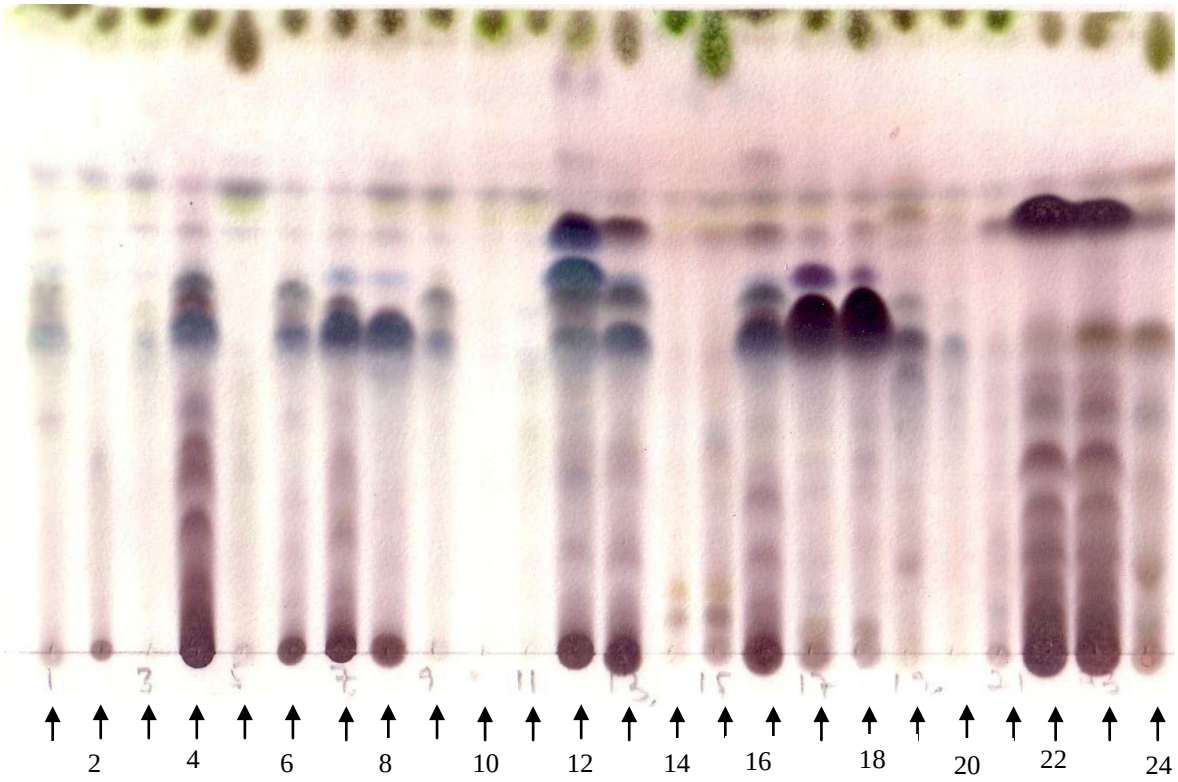

Figure 4. TLC profile of Loranthaceae specimens sprayed with Gibbs reagent.

Loranthaceae sp. using Gibbs, vanillin-sulphuric acid and Dragenddoff spray reagents for TLC are shown in Figures 4, 5 and 6, respectively. In Figures 4, 5 and 6, Gibbs reagent were used for visualizing phenolics, vanillin in sulphuric acid for terpenoids, while Dragendoff reagent was used to see if alkaloids were present on the TLC plate, respectively. Table 2 shows the R_f values of spots found on the TLC plate in Figure 4, which reveals that all the specimens had phenolics in them although to varying degree judging from the numbers of spots. *T. cordifolius* on *Cassia* sp. from Jos (spot 12), *G. braunii* on *P. americana* (spot17) and *G. braunii* (spot 18) from Calabar have the highest number of spots of 13, 13 and 12, respectively (Table 2). An intermediate number of spots were found in *T. globiferus* on *P. curattelifolia* from Kano (spot 4), *P. nigritana* on *Citrus* sp. from Suleija (spot 16) and *G. braunii* on *Cola* sp. from Ibadan (spot 19) with 10, 11 and 11 spots respectively (Table 2). The lowest spots are found in *T. globiferus* on *A. indica* (spot 2), *T. globiferus* on *Tectona grandis* (spot 3), *T. globiferus* on *Zyzyphus* sp. (spot 5), *T. bangwensis* on *Newboldia laevis* (spot 10) and *Agelanthus dodoneifolius* on *P. biglobosa* (spot 21) with 3, 5, 3, 3 and 5, respectively (Table 2). Spots with R_F values of 0.29 - 0.32 and 0.71 - 0.73 are found to be present in over 90% of the specimens. In Figure 5, terpenoids were only observed in some of the specimens. Alkaloids were absent from all the specimens studied (Figure 6).

DISCUSSION

The phytochemical analysis and the TLC profiling showed variation in the constituent secondary metabolites among various species irrespective of their host and ecological location (Table 1; Figures 4 and 5). Variation in secondary metabolites among the same mistletoe species occurring on different host plants have been observed in earlier studies (Deeni and Sadiq, 2002; Ibrahim et al., 2009). The only consistent pattern from this study was the lack of alkaloids from all the specimens analyzed (Table 1; Figures 1, 2 and 6). It is a known fact that quantitative and qualitative information on secondary metabolites is useful for taxonomic classification of plants (Harborne, 1968; Takhtajan, 1973). Hence absence of alkaloids, and the number of species testing positive for balsam and phenols appears to be of chemotaxonomic significance among the species in Nigeria. Alkaloids were not recorded for any of the Nigerian Loranthaceae specimens studied but Sanchez-Areola et al. (2004) recorded the presence of alkaloids in *Psittacanthus calyculatus,* a New World Loranthaceae endemic to Mexico (Kuijt, 2009).

The TLC R_f in Table 2 shows that there were similar phenolic compounds (R_f values of 0.29 - 0.32 and 0.71 - 0.73) present in most of the specimens, over 90% of the species and this further reinforced the fact that phenolics could be a source of analytical marker compound(s) for

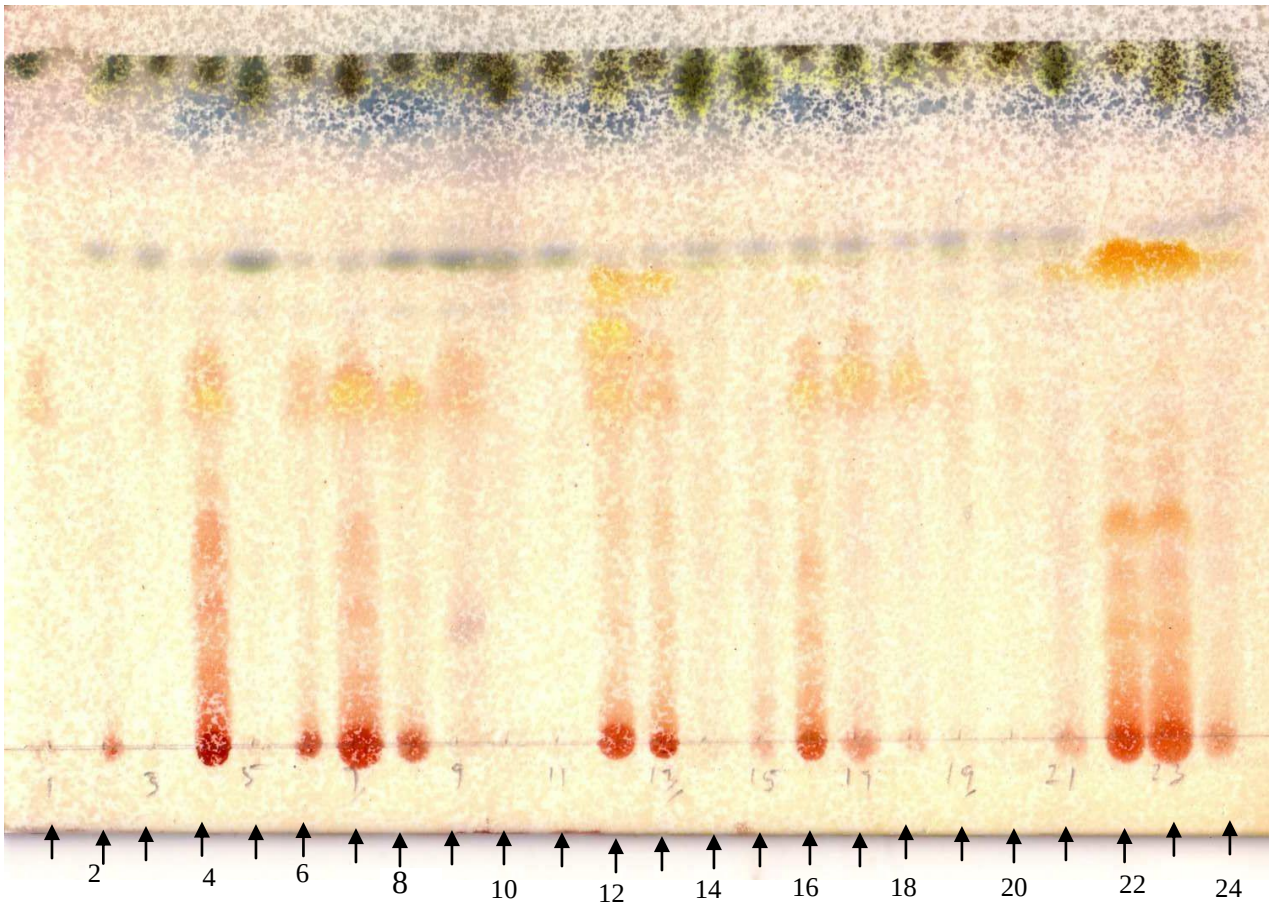

Figure 5. TLC profile of Loranthaceae specimens sprayed with Vanillin-sulphuric acid reagent.

standardization of herbal preparations from these species. High amounts of phenolics have long been known to be a phytochemical feature of parasitic flowering plants and they are said to occur at a level that is generally higher than the host plant (Khanna et al., 1968; Salatino et al., 1993). The study reveals that *G. braunii* specimens irrespective of their hosts or locations are rich in phenolic compounds as compared to other species while *T. globiferus* and *T. bangwensis* are depauperate in phenolics as compared to other species. Also of note is the absence of glycosides in the *Phragmanthera* species and total absence of tannins from all the specimens from Ibadan. These findings may be of chemotaxonomic importance. Thus, the presence of balsams and phenols could be used in specific combination with morphological characteristics and bio-geographical distribution ranges for the delineation of genera and species in the family (Crockett and Robson, 2011).

Research on dwarf mistletoes (Viscaceae) in North America indicates that plant chemistry, particularly secondary metabolites, plays an important role in determining interactions between host and parasite (Snyder, 1996). This may not be applicable to Nigerian Loranthaceae because of the variation noted in the metabolites present in the same species on different hosts. Differences in chemical profiles of the various species studied underscore why the specific choice of species for the treatment of a particular ailment is very important. This study has shown that some species may not possess a particular metabolite that is common in other species. For instance, the absence of glycosides in *Phragmanthera* species or tannins and saponins in *T. bangwensis* may result in major pharmacological differences. Although the correlation between host and chemical profile of the species was not clearly defined in this study, it is believed that the host could play a role in the observed chemical profile of the plant or species. The influence of host chemistry on the chemical constituents of the parasite on different hosts might justify why the host is as important as the parasite in pharmacognosy, ethnopharmacology and ethnomedicine, and why the use of these Loranthaceae in the treatment of an ailment is often dependent on a particular or specific host (Burkill, 1995; Snyder 1996; Adodo, 2002; Olapade, 2002; Preston et al., 2010), for instance, in Brazil, where there is preference for *Cladocolea micrantha* growing on cashew tree (*Anacardium occidentale*) for the treatment of tumors

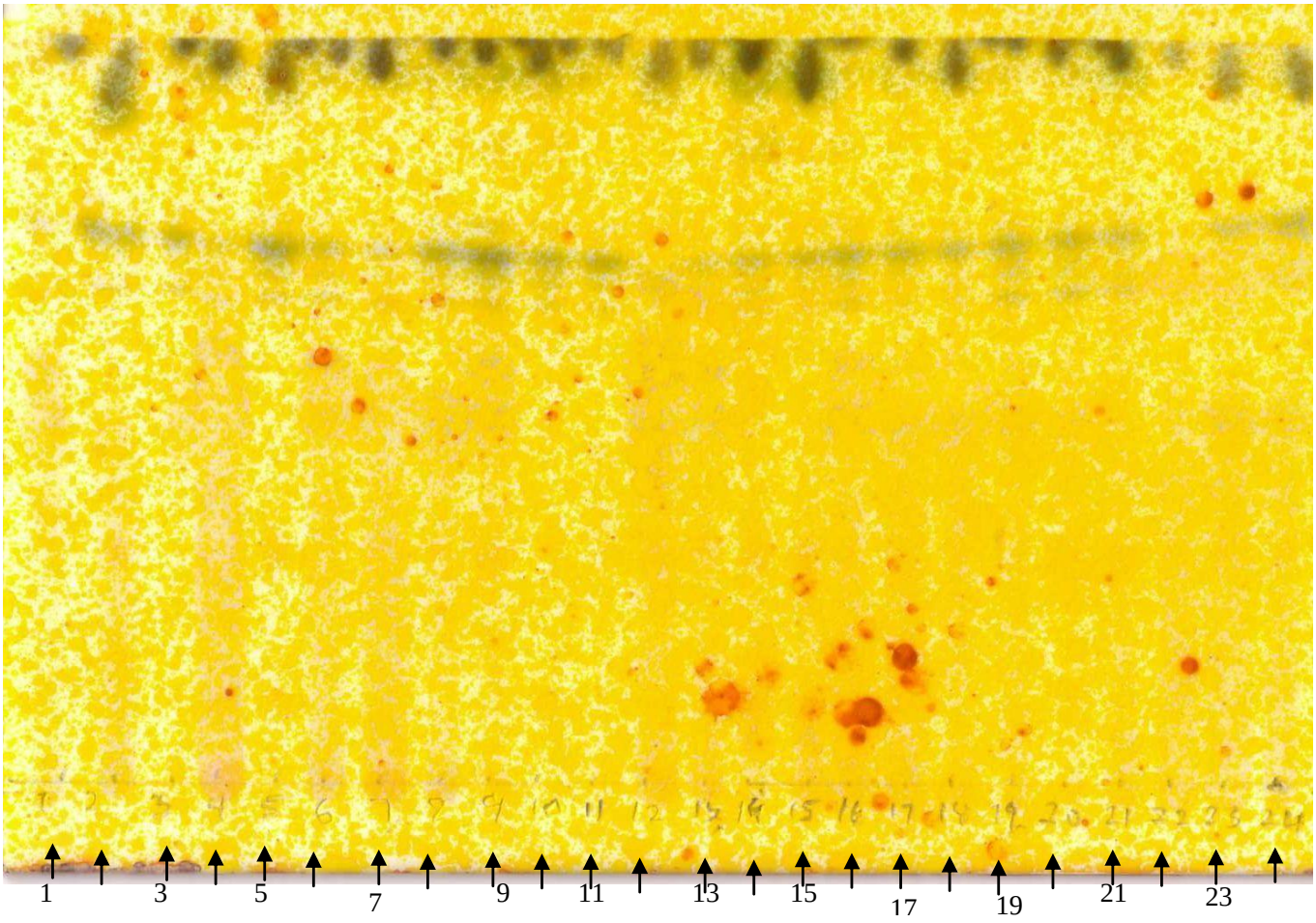

Figure 6. TLC profiling of Loranthaceae specimens sprayed with Dragendoff reagent.

and inflammatory diseases (Adodo, 2002; Olapade, 2002; Guimaraes et al., 2007).

Conclusion

From this investigation, species of Loranthaceae in Nigeria might not be delineated by scoring presence or absence of their secondary metabolites qualitatively or quantitatively due to variations which occur on same species form different hosts but the occurrence of similar metabolites like phenolics and balsam in most, if not all the species irrespective of the host and locality is useful taxonomically as a marker for the group. It is therefore our recommendation that caution should be exercised in the use of Loranthaceae as phytomedicine because of the chemical variations which exist in the same species found on different hosts. The same species collected from two different hosts might have different pharmacological effects in the body. The group is currently working on determining a phytochemical marker for the family Loranthaceae in Nigeria.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Short Communication

Efficacy of leaf extracts of some medicinal plants on growth of *Colletotrichum capsici* butler and bisby

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Attempts were made to determine the effect of leaf extracts of *Azadirachta indica, Ocimum sanctum*, *Tridex procumbens*, *Clerodendron innermis, Cathranthus roseus, Ricinus communis, Citrus limon* against *Colletotrichum capsici*. Out of these medicinal plants tested, 15% alcoholic extract of *Azadirachta indica and O. sanctum* was found inhibitory for the growth of *Colletotrichum capsici.* The results show that extracts from leaves of different plants vary in their effects on growth of *C. capsici*. It is evident from the results that aqueous and alcoholic leaf extracts of *A. indica* and *O. sanctum* exhibited strong fungitoxicity against *C. capsici.* Alcoholic extract of all the seven plants showed significant result as compared to aqueous extracts.

Key words: Chilli, anthracnose, leaf extract, *Colletotrichum capsici*.

INTRODUCTION

The total area under the cultivation of chilli crop in India is about 0.7 to 0.9 million hectars. It is grown extensively in Tamil nadu, Andhra pradesh, Karnataka and Maharashtra. It is grown as a rain fed crop in most parts of Andhra pradesh and as an irrigated crop in other areas. There are several varieties of chilli grown in India and some are non-pungent with large sized fruits that are used mainly as vegetables. Chilli is one of the important crops grown for its valuable fruit in making spices and condiments. It forms a part of the Indian diet. The fruits are used either dry or raw. It is used in green as well as dry powder form, rich source of vitamin A and vitamin C among the vegetable.

In chilli, there are various types of diseases but anthracnose is a serious disease of chilli found in India, caused by *Colletotrichum capsici* where it occurs in severe form in all the southern states. The disease is developed due to hot and humid conditions. The disease has been identified in all the chilli producing regions of the world and has become a serious constrain in chilli production whenever the crop is grown. Anthracnose causes extensive pre- and post-harvest damage to chilli fruits causing anthracnose lesions. These fungal infection are known to cause heavy damages and impair the quality of fruit seeds. Even small anthracnose lesions on chilli fruits reduce their marketable value (Manandhar

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et al., 1995). Anthracnose disease can occur on leaves, stems and both pre- and post-harvest fruits (Isaac, 1992). Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses under severe disease pressure, lesions which may coalesce. Conidial masses may occur in concentric rings on the lesions. Management and control of the anthracnose disease are still under extensive research (Yoon et al., 2004). Many studies have concluded that disease management practices are often inadequate to eliminate the diseases. Breeding to develop the long-lasting resistant varieties has also not been successful due to involvement of multiple *Colletotrichum* species in anthracnose infection.

MATERIALS AND METHODS

Collection of samples

Diseased chilli fruits were collected in polyethylene bags from fields and local market of Nanded city of Maharashtra State (India).

Identification of pathogen

The diseased chilli fruits were preliminary observed for sporulation characters like asexual or sexual spores or fruiting structures under compound microscope and their Identification was confirmed with the help of latest manuals (Subramanian, 1971; Jha 1993). Pure cultures of the identified fungus was prepared and maintained on Czapex dox agar slants for further experiments.

Preparation of plant extracts

Seven common and easily available plants like *Azadirachta indica, Ocimum sanctum, Tridex procumbens, Clerodendron innermis, Cathranthus roseus, Ricinus cummunis, Citrus limon* were selected. The leaves of the plant were collected separately, surface sterilized with 0.1% $HgCl_2$ and washed repeatedly with sterile distilled water for several times and kept for drying in hot air oven (Metalab) at 60°C temperature for 48 h.

Aqueous extract

The dried leaves of selected plants were crushed separately into fine powder with the help of blender 5, 10 and 15 g each of the plant powder was dissolved separately in 100 ml sterilized hot distilled water and filtered through Whatman No.1 filter paper. The filtrates were used as 5, 10 and 15% concentrations of aqueous plant extracts, respectively.

Alcoholic extracts

For alcoholic extract 5, 10, 15 g of each sun-dried medicinal plant material, were cut into small pieces and then macerated by blender 1 to 2 mm separately and the powder produced was blended in ethyl alcohol (1:10 w/v) and extracted under cold conditions for 24 h. The resultant extract was filtered through a glass wool filter and then rinsed with a small quantity (30 ml) of 96% alcohol. The extracts were evaporated under reduced pressure at 40°C. Subsequently, the extracts were diluted by distilled water and stored in the deep freezer at -10°C (Fardos, 2009).

Evaluation of plant extracts against *C. capsici*

The effect of 5, 10, and 15% aqueous and alcoholic leaf extract was determined by measuring the mycelial dry weight. 50 ml of glucose nitrate medium was poured into each flask containing different concentrations (5, 10 and 15%) of the respective extracts (2 ml each). With a sterile cork borer (3 mm), mycelial disc of seven days old cultures of the isolates were inoculated in the flask and incubated at 28 ± 2°C. After seven days, the content of flasks were filtered through Whatman No. 1 filter paper The content were dried at 70°C for 24 h and percentage inhibition of mycelial growth was evaluated using the poisoned food techniques (PFT), and calculated using the formula given by Vincent (1927) and Ogbebor et al. (2007).

$$\% \text{ Inhibition} = \frac{100 (\text{Control} - \text{Treatment})}{\text{Control}}$$

RESULTS AND DISCUSSION

Biological control of fruit rot and dieback of chilli with plant products tested in many laboratories and field trials showed that the *O. sanctum* leaf extract and neem (*A. indica*) oil could restrict growth of the anthracnose fungus (Jeyalakshmi and Seetharaman, 1998). It is clearly evident from the results that the aqueous and alcoholic leaf extracts of all the plants tested against *C. capsici* significantly reduced mycelial dry weight, leaf extract of *A. indica* showed high percentage inhibition of mycelial dry weight (60.62, 71.05 and 81.57%) at 5, 10 and 15% aqueous extract while leaf extract of *Ricinus cummunis* showed very low percentage inhibition of mycelial dry weight (25, 37.36 and 35.52) (Tables 1 and 2). The alcoholic leaf extracts of all the plants tested were found to be more effective as compared to aqueous leaf extracts. The leaf extract of plants which vary in their effect on growth of *C. capsici* may be due to differential effect of active ingradient present in plants. Percentage inhibition of mycelial dry weight of *C. capsici* was highly inhibited in 15% alcoholic leaf extract of *A. indica* followed by leaf extract *O. sanctum.* Upadhyaya and Gupta (1990) reported the control of *Curvularia lunata* with extracts of *Ocimum sanctum*. Singh et al. (1993) reported the effectiveness of aqueous extracts of *O. sanctum* and *A. indica* in the control of disease development in banana. In this study, the differences in the inhibition of mycelial growth of *C. capsici* may be due to variations in fungitoxicity of leaf extract. Similarly, Kurucheve et al. (1997) observed that the variation in the inhibitory effect of plant extracts may be due to qualitative and qualitative differences in antifungal principles. The strong fungitoxicity exhibited by the leaf extract may be due to presence of chemical constituents including tannins, glycosides, alkaloids and flavonoids (Harborne, 1984).

It is clear from the results that all the leaf extracts of seven plants exhibited antifungal activity. Among these, many

Table 1. Effect of aqueous leaf extracts of some medicinal plants on *C. capsici.*

Plant name	% inhibition of mycelial dry weight at different concentrations		
	5%	10%	15%
Azadirachta indica A. Juss.	60.62	71.05	81.57
Ocimum sanctum L.	51.31	65.78	76.31
Clerodendrum inerme (L.) Gaertn.	35.52	50.00	53.94
Tridax procumbens L.	44.73	57.89	68.42
Catharanthus roseus (L.) G. Don	42.10	55.26	63.15
Ricinus communis L.	25.00	37.36	35.52
Citrus limon L.	31.57	42.89	46.05

Table 2. Effect of alcoholic leaf extracts of some medicinal plants on *C. capsici.*

Plant name	% inhibition of mycelial dry weight at different concentrations		
	5%	10%	15%
Azadirachta indica A.Juss.	68.42	76.31	89.47
Ocimum sanctum L.	63.15	75.00	84.21
Clerodendrum inerme(L.) Gaertn.	53.94	68.42	72.36
Tridex procumbens L.	57.89	72.36	76.31
Catharanthus roseus (L.) G.Don	54.47	71.05	75.00
Ricinus communis L.	50.00	62.63	67.36
Citrus limon L.	52.63	65.26	71.05

workers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides (Tewari, 1995; Lakshmanan, 1990; Singh et al., 1993; Ogbebor et al., 2005).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effects of crude extracts on some selected physiological parameters of French beans (*Phaseolus vulgaris*) infected with rust (*Uromyces appendiculatus*)

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Rust (*Uromyces appendiculatus*) is a major foliar disease that reduces yield and pod quality in beans. The field trial of French beans was established at Jomo Kenyatta University of Agriculture and Technology (JKUAT). Single plant extracts and combinations of *Boscia angustifolia, Zanthoxylum chalybeum* and *Melea volkensii* were used to evaluate their effect on *U. appendiculatus* in the field. During the growing period, beans were infected with rust from natural inoculum at the field. Physiological responses such as carbon dioxide assimilation, transpiration (E), stomatal conductance (gs), and photosynthetic rate (Pn) of French beans treatments were examined after extracts of three antifungal plants were sprayed. *B. angustifolia - Z. chalybeum* combination and single plant treatment *M. volkensii* had positive effects on enhancing the rate of photosynthesis in bean plants. The high regressions between stomatal conductance and rate of transpiration in the treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. These plant extracts however caused an increase in the rate of transpiration of the bean plants, which resulted in loss of water. Results reveal bioactive potential of the flora from *M. volkensii* and a combination of *B. angustifolia* and *Z. chalybeum* to produce metabolites with potential applications as botanical pesticides.

Key words: Antifungal, beans, physiological responses, rust.

INTRODUCTION

The importance of the French beans is due to their high nutritive value in both energy and protein contents. Therefore, increasing the crop production is one of the most important targets of agricultural policy in several countries. The bean rust fungus (*Uromyces appendiculatus*) is of worldwide importance as a yield-reducing disease of *Phaseolus vulgaris* L., potentially cause yield losses up to 50% (Venette and Jones, 1982; Berger et al., 1995; De Jesus Junior et al., 2001). Under severe disease, it completely defoliates the plant and can cause 100% crop failure (Steadman et al., 2002). Rust result in harmful effects on growth, most physiological

activities and the yield of beans. On global scale, studies have shown that some plant species have antifungal compounds (Fabry et al., 1996; Okemo et al., 2003). Within this context, natural products from plants seem to be a good alternative since numerous plants have the potential to control phytopathogenic fungi, and have much prospect to be used as a fungicide. Despite the many studies performed on biological control, relatively little is known about the role of the plant extracts (*Boscia angustifolia, Melea volkensii* and *Zanthoxylum chalybeum)* applied on the physiological parameters of the plants. In this study, we hypothesized that antifungal plant extracts might influence physiological activities of bean plants. Therefore, this study aimed at studying the role of selected plant extracts (added singly or in combination) in influencing photosynthetic activities of bean plants and finding an explanation for the above role based on test attributes.

MATERIALS AND METHODS

Study site

Field studies were carried out at JKUAT in Thika District. The university is located at latitude 1°05 S and longitude 37°00 E. It lies at an altitude of 1525 m above sea level and it receives an annual rainfall of 850 mm. Temperatures range from 13 and 26°C.

Collection and processing of plant materials

The samples of desired plants (*B. angustifolia, M. volkensii* and *Z. chalybeum)* from previous experiments (Omwenga, 2009; Kiswii, 2009) for antifungal activity were collected from different parts of the country (Samburu, Mombasa, Mwingi, Kakamega forest and Nakuru) in clean sacks (Table 1). The plants were identified and verified at Jomo Kenyatta University of Agriculture and Technology (Taxonomy unit, Department of Botany). Voucher specimens were deposited in the herbarium. The samples were labeled and deposited in the botany laboratory. The plant leaves and roots were dried separately at room temperature for a period of 1-2 weeks and then ground separately to powder using a grinding mill at 8000 rpm (Type 8 lab mill). The powder was stored in plastic bags at room temperature until the time required. Two kilograms of each plant sample was soaked and left overnight to allow extraction of the crude active compounds. The supernatant was filtered in several layers of muslin cloth and volumes adjusted to 20 L. (Stoll, 2000). A combination of *B. angustifolia, M. volkensii* and *Z. chalybeum* extracts was used because previous experiments (Menge, 2011) revealed a better synergism between the two in reducing the disease severity. A normal washing bar soap ground to powder and dried was used as a sticker at a rate of 1 g per litre of water extracts. Untreated control was used containing water and soap only without the extracts. During the growing period, beans were infected with rust from natural inoculum at the field. Seeds were obtained from Regina Seed Company and planted at a spacing of 30 cm between rows and 10 cm between plants within the rows (Monda et al., 2003). French bean seeds commercially available coated with thiram were used to control root rots. French bean variety Amy seeds were planted in 4 × 3 m plots each separated by a 1 m path between the treatments and the replications. Amy is high yielding as compared to other varieties therefore it is grown by most farmers. Di-ammonium phosphate was used at planting, at a rate of 200 kg/ha mixed well before seed placement. Calcium ammonium nitrate was applied at a rate of 100 kg/ha at trifoliate leaf stage.

Experimental design type

The experiment was carried out in a randomized complete design, and data analyzed using analysis of variance; and comparison of means was made by using Duncan's multiple range test. The treatments consisted of six plant extracts, copper hydroxide 61.4% (Kocide DF: metallic copper equivalent 40% formulated as a dry flowable) as a positive control and a negative water control. A spray regime of once a week using a knap sack was employed from the fifteen days after planting until flowering. The extracts were used as protectants. The fungicide was applied at a rate of 2.5 kg ha^{-1} according to the manufacturers' recommendations. There were a total of seven hundred and sixty plants per replicate. Overhead irrigation twice a week and weeding were done as necessary.

CO_2 exchange measurements

Three different types of leaf gas exchange measurements were made on plants from the interior rows of the plots. First, at approximately weekly intervals, measurements of carbon dioxide assimilation rate were made at 0900, 1200, and 1500 h at the JKUAT farm. Mature, fully illuminated upper canopy leaves were measured at their nominal daytime growth. Daylight patterns of carbon dioxide assimilation rate were measured by the infrared gas analyzer (IRGA). IRGA was used as a null point instrument that allows the flow of carbon dioxide into the system at a rate equivalent to the rate of uptake of the leaf. The amount of carbon dioxide assimilated by the leaf was read directly from the IRGA. French bean leaf tissues from ten selected plants from each treatment were enclosed in the leaf chamber (leaf chamber = 2.5 cm^2) one at a time. The air flow rate through the chamber remained fixed. The carbon dioxide assimilation was monitored for 1 min for each leaf by the IRGA connected in an open gas flow system. During measurement of CO_2 assimilation rate the following parameters were also recorded using IRGA; stomatal conductance, and transpiration. IRGA determines the rates of photosynthesis (AN) and transpiration (E), as:

$AN = (\text{Air flux} \times \Delta CO_2)/\text{Leaf area}$

$E = (\text{Air flux} \times \Delta H_2O)/\text{Leaf area}$

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Abbreviations: PAR, Photosynthetic active radiation; **C_3,** carbon parameters; **JKUAT,** Jomo Kenyatta University of Agriculture and Technology; **Pn,** photosynthetic rate; **IRGA,** infrared gas analyzer; **E,** transpiration; **gs,** stomatal conductance; **Cu^{2+},** copper; **kg/ha,** kilograms per hectare.

Table 1. Selected antifungal plant extracts for the study and parts of the plants used.

Family	Scientific Name	Common/local name	Parts used
Capparidaceae	*Boscia angustifolia*	Mulule (Kamba)	Leaves, stem
Rutaceae	*Zanthoxylum chalybeum*	Mjafari (Swahili)	Leaves, stem
Rutaceae	*Melea volkensii*	Mukau (Kamba)	Leaves, stem

From Fick's first law of diffusion, other parameters (stomatal conductance and CO_2 assimilation rate) were then calculated.

RESULTS

Stomata conductance (gs) and transpiration rate

The diurnal changes in gs, rate of transpiration and photosynthetic rate under the antifungal treatments are as shown in Figures 1a and b. The stomatal conductance in Figure 1a followed the same pattern in all the treatments being highest at 9:00 am, dropped at midday and maintained low levels in the late afternoon. However, there were significant differences in stomatal conductance (P=0.0173) in the treatments at 9:00 am and was rated as *B. angustifolia - Z. chalybeum* (77.7 mol/$m^{-2}sec^{-1}$) combination having the highest stomatal conductance followed by, *M. volkensii* (46.3 mol/$m^{-2}s^{-1}$) and Kocide DF (39.18 mol/$m^{-2}s^{-1}$), respectively. *M. volkensii* plant extract showed lowest stomatal conductance as compared to the other plant extract combination. The stomatal conductance for commercial fungicide (Kocide DF) was significantly lower (25.6 mol/$m^{-2}s^{-1}$) than other treatments at 12:00 pm. *B. angustifolia - Z. chalybeum* (41 mol/$m^{-2}s^{-1}$), *M. volkensii* (41.5 mol/$m^{-2}s^{-1}$) and untreated control (38.3 mol/$m^{-2}s^{-1}$) were not significantly different from each other at 12:00 pm. There were no significant differences in stomatal conductance at 15:00 pm of all treatments (P=0.1235). This showed that apart from controlling fungal attack the treatments had some influence on stomatal conductance. This behavior was observed in all the four treatments in the three months growth period. Figure 1b shows the diurnal changes in the rate of transpiration in the four treatments. The rate of transpiration was highest at 9:00 am coinciding with highest stomatal conductance and dropped at noon when stomatal conductance also dropped and maintained low levels in the early afternoon and evening when stomatal conductance and PAR were low. There were significant differences (P=0.003) in transpiration rates of the treatments at 9:00 am. *B. angustifolia - Z. chalybeum* (2.065 mol/m^2/s) and *M. volkensii* single plant extracts (1.353 mol/m^2/s) had significantly the highest rate of transpiration as compared to other treatments. There were significant differences (P=0.0015) in the rates of transpiration among the treatments at 12:00 pm. Kocide DF (0.76 mol/m^2/s) had significantly the lowest rate of transpiration while there were no differences in *B. angustifolia - Z. chalybeum* (1.12 mol/m^2/s), *M. volkensii* (1.135 mol/m^2/s) and untreated control (1.067 mol/m^2/s). There were significant differences (P<0.05) in transpiration rates of the treatments at 15:00 am. *B. angustifolia - Z. chalybeum* (0.67 mol/m^2/s), *M. volkensii* (0.78 mol/m^2/s) and Kocide DF (0.77 mol/m^2/s) treated beans had lower rates of transpiration at 15:00 pm than the untreated control (1.3 mol/m^2/s) at 15:00 pm. Generally both the single and combinations of plant extracts had a positive effect on the rate of transpiration. The high positive regressions (r^2 >0.9) and the regression equations are summarized in Table 2.

Effect of treatment on CO_2 assimilation and photosynthetic rate (Pn)

Figures 1 and 2 show diurnal changes in photosynthetic (Pn) and CO_2 assimilation rates in the four treatments, respectively. The more the negative CO_2 assimilation the more CO_2 is absorbed from the environment as shown in Figure 2. The CO_2 assimilation reached a peak at 9:00 am and decreased sharply at noon and eventually maintained low levels in the afternoon. CO_2 assimilation followed the same pattern as that of stomatal conductance. There were significant differences (P<0.001) in CO_2 assimilation rates among treatments at 9:00 am. *B. angustifolia - Z. chalybeum* (577.933 ppm) treated bean plants had significantly lowest CO_2 assimilation rate while there were no differences between *M. volkensii* (679.5 ppm), Kocide DF (641.364 ppm) and untreated control (651.154 ppm) in CO_2 assimilation rate at 9:00 am. There were no significant differences (P>0.002) in CO_2 assimilation rate of all treatments at 12:00 pm however they ranged from untreated control (362 ppm) being the highest then followed by *B. angustifolia - Z. chalybeum* (328.33 ppm), *M. volkensii* (320.33 ppm) and Kocide DF (304.18 ppm), respectively. Likewise, at 15:00 pm there were no differences (P=0.1425) in CO_2 assimilation rates of all treatments. The relationship between stomatal conductance and CO_2 assimilation was described by low insignificant positive regressions in each treatment as shown in Table 3. The low R^2 indicated the two parameters were very slightly interrelated. The diurnal pattern of rate of photosynthesis among the treatments was the same being highest at the morning, 9:00 am dropped at noon and remained low in the afternoon (15:00 pm). There were significant

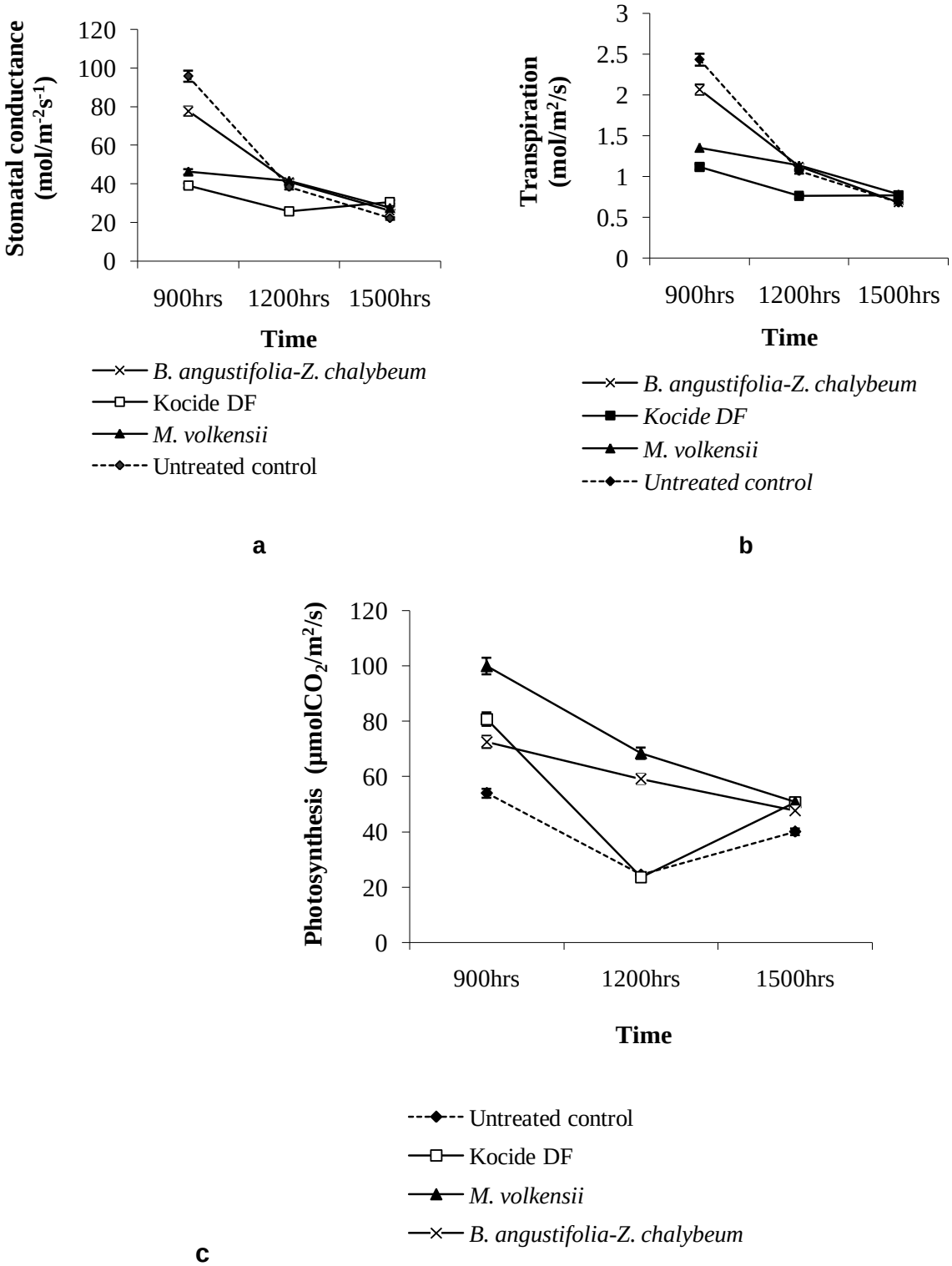

Figure 1. Daily diurnal courses of stomatal conductance (a), rate of transpiration (b) in French beans (Amy variety) exposed to various treatments. Each point represents the mean ± standard error of six replications.

differences (P=0.0021) in the rate of photosynthesis among the treatments at 9:00 am. The rate of photosynthesis was rated highest in *M. volkensii* (99.9 µmolCO_2/m^2/s) as compared to the combination *B. angustifolia - Z. chalybeum* (72.5 µmolCO_2/m^2/s) and untreated control (53.9 µmolCO_2/m2/s), respectively.

Table 2. The relationship between stomatal conductance and rate of transpiration among the four treatments.

Treatment	Equation	R^2
B. angustifolia - Z. chalybeum	y =44.851x -10.454	0.9829
Untreated control	y = 41.604x -4.882	0.9656
Kocide DF	y = 38.824x -3.8036	0.9449
M. volkensii	y = 37.407x -0.7395	0.9396

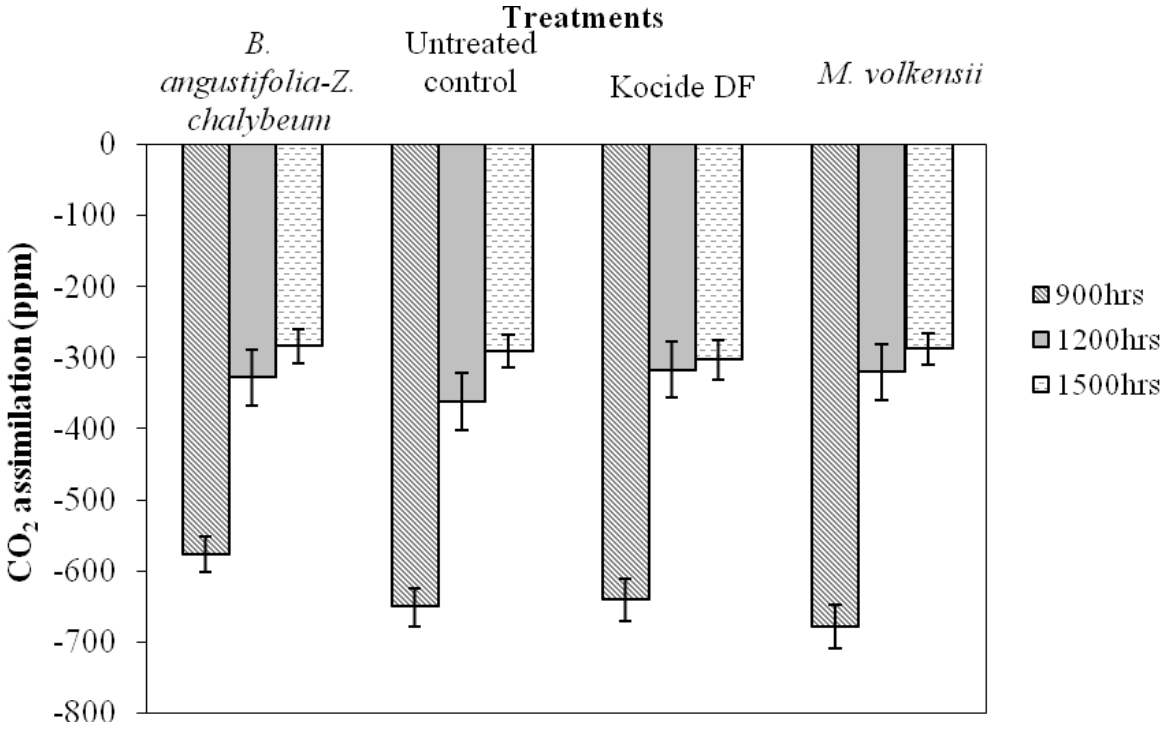

Figure 2. Daily courses of CO_2 assimilation in French beans exposed to various antifungal plant extracts and a commercial fungicide under natural conditions.

Table 3. Linear relationships between CO_2 and the rate of photosynthesis.

Treatment	Equation	R^2
B. angustifolia - Z. chalybeum	y = 4.0963x + 74.228	0.5873
Untreated control	y = 0.0369x + 39.852	0.0801
Kocide DF	y = 0.1134x + 7.1438	0.4250
M. volkensii	y = 0.0994x + 27.677	0.6596

There were significant differences (P=0.0132) in the rate of photosynthesis among the treatments at 12:00 pm. *M. volkensii* (68.38 µmolCO_2/m^2/s) had significantly the highest photosynthetic rate at 12:00 pm followed by *B. angustifolia - Z. chalybeum* (59.1 µmolCO_2/m^2/s). However, there were no differences between Kocide DF (23.51µmolCO_2/m^2/s) and untreated control (24.4 µmolCO_2/m^2/s) at 12:00pm. *M. volkensii* (50.77 µmolCO_2/m^2/s) and Kocide DF (50.7 µmolCO_2/m^2/s) revealed significantly the highest rates of photosynthesis although they were not different from each other at 15:00bpm. Untreated control (39.98 µmolCO_2/m^2/s) had the lowest photosynthetic as compared to other treatments at 15:00 pm. Generally, *M. volkensii* had a positive effect on rate of photosynthesis as compared to other *B. angustifolia - Z. chalybeum.*

DISCUSSION

Effect of treatment on some selected physiological parameters

Generally, French bean leaves showed higher values of stomatal conductance with consequent higher transpiration. The high positive regressions (r^2 >0.9) were obtained in the four treatments. This indicated that stomatal conductance and rate of transpiration were interdependent and it is interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day.

The response of stomata to transpiration was used by Monteith (1995a), who re-analyzed 52 sets of published measurements at canopy scale of humidity responses on 16 species of monocots in terms of the relation between stomatal conductance and transpiration. *B. angustifolia* - *Z. chalybeum* combination and single plant treatment *M. volkensii* had a positive effect on stomatal conductance of bean plants. This suggests that these antifungal plant extracts in general may have interfered with any one of the several biosynthetic pathways or energy production pathways. Commercial control (Kocide DF) had the lowest stomatal conductance of all treatments however; Kocide DF plots had the lowest water loss as compared to others, this indicates they were better at water conservation. Stomata showed a slight opening tendency until 1200 noon, when decreases in stomatal conductance were likely cut down in high transpiration (E) values. Since similar stomatal conductance values were observed during morning, changes in E values suggest that stomatal aperture was more than sufficient to support maximal E values since early hours of morning.

The high regressions between stomatal conductance and rate of transpiration in the four treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. The differences in R^2 values in four treatments were insignificant meaning that concerning these two parameters, the French beans responded to the treatments the same way. This pattern was maintained throughout the growing period. Therefore, the sources of variation in stomatal conductance and the rate of transpiration were treatment, time.

The daily diurnal courses conformed to Zeiger et al. (1981) study which showed that at dawn, stomatal conductance usually increases very rapidly because the entrained rhythm is in correct phase, and also there is a great sensitivity to low photon fluxes of blue light at this time. Stomatal conductance then increases gradually towards a maximum value in late morning or early afternoon before declining noticeably later in the day. This partial closure in the afternoon is thought to be driven by the entrained rhythm, and it is not unusual for the stomata to be nearly closed before dusk. The responsiveness of stomata to light and CO_2 depends on leaf age and past treatment. As leaves become older, the stomata often become less responsive and may open partly, even at midday. It is difficult to generalize stomatal behavior because so many contradictory reports occur in the literature. Stomatal activity is affected by numerous internal and external factors which often interact in complex ways that sometimes are overlooked by investigators.

Daily course of CO_2 assimilation was similar for all evaluated treatments. In early morning, the sharp increase in photosynthetic photon flux density seems to be the main cause of CO_2 assimilation increase. Considering the highest CO_2 assimilation values, no significant difference was found between treatments under natural condition. Maximal CO_2 assimilation rates were reached around 9.00 am until 12:00 pm when reductions were recorded. Low stomatal conductance is known to cause decrease in CO_2 assimilation values by reducing the CO_2 available, which may be indicated by decreased intercellular CO_2 concentration values (Jones, 1998; Nobel, 1999). Commercial control (Kocide DF) treated plants had the lowest carbon dioxide released as compared to all other treatments because of its low stomata conductance.

Photosynthetic rates (Pn) among the four treatments followed a trend whereby they were at the peak at 9:00 am reducing gradually towards the afternoon and at 15:00 pm. The main sources of variation in the Pn might have been due to treatment and the time of the day. The explanation for the above stated interactions being significant could be that these factors were affecting the photosynthesis rates dependently. The antifungal plant extract had a positive effect on the rate of photosynthesis than other treatments. Therefore, the results suggest that the photosynthetic capacity of 'commercial control (Kocide DF) treated beans' were constrained at natural condition by low stomata conductance. Low stomata conductance in the commercial control (Kocide DF) treated plants might have affected the photosynthetic activity. The inactivation of Rubisco (ribulose-bisphosphate carboxylase/oxygenase) a key-enzyme of Calvin cycle and its two accompanying enzymes, that is, Rubisco activase and carbonic anhydrase under the stress conditions caused by copper and lead (not examined) may be regarded as another possible factor (Vojtechova and Leblova, 1991). This indicated that plant extract treatments were leaf physiology friendly as compared to the copper containing Kocide DF. The untreated control highest transpiration rates might have been caused by high disease severity. Rust caused increased transpiration (E) from infected tissues after sporulation in untreated control. Transpiration before sporulation, which potentially is by a mainly stomatal pathway, is inhibited, probably by stomatal closure; rust is known to inhibit stomatal opening in the light in other

diseases, e.g. bean (*P. vulgaris*) infected by either *U. phaseoli* (Duniway and Durbin, 1971b) or *Uromyces appendiculatus* and this effect has recently been confirmed for Faba bean rust (Tissera, unpublished results). In the present study, it was noted that at each sample time, more variability in transpiration rate occurred in rusted tissue than in healthy tissue. This variability probably occurred because the number of lesions per unit area of leaf was not controlled. Durbin (1978) stated that when sporulation occurred, transpiration from bean leaves infected with rust increased by as much as 50%. Where net photosynthesis was concerned, infection induced opposing changes in the four treatments; net photosynthesis in healthy leaves increased because gross photosynthesis was stimulated and photorespiration was inhibited. Net photosynthesis per plant and ultimately plant growth of the untreated control reduced because infection inhibits the growth of leaf area.

Photosynthesis is closely related to crop growth and yield, and higher photosynthetic rate of leaves is one of the important factors for high crop yield. The results showed that after flowering, the leaves gradually aged, the net Pn, E and gs of leaves gradually declined. Commercial control (Kocide DF) contains copper metal that might have caused low productivity. This could be attributed to its contents that can hamper the process of photosynthesis. It being a micronutrient, copper improves plant growth at natural concentrations. However, at higher concentrations, it also proves very toxic to plants. The phytotoxic effects related to higher concentrations of copper include inhibition of photosynthetic efficiency and as a result reduced crop productivity. The process of photosynthesis was adversely affected by Cu toxicity. Plants exposed to copper formulated fungicide (Kocide DF) showed a decline in photosynthetic rate, which might have resulted from distorted chloroplast structure, restrained photosynthesis of chlorophyll and carotenoids, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closure (Vojtechova and Leblova, 1991).

A strong relationship exists between Kocide DF application and a decrease in photosynthesis and it is believed to result from stomatal closure. Increased rates of respiration and loss of chlorophyll from the leaf tissue apparently were the major factors responsible for the reduction of photosynthetic rates on diseased untreated control leaves.

The photoinhibition mechanism could have a character of photoprotection or represent damaging in PSII reaction centers (Osmond, 1994). The maximum CO_2 assimilation values observed are in agreement with the measurements performed by Souza et al. (2003) in common bean study. Transpiration exhibited similar trend to photosynthesis suggesting that an appreciable part of the inhibition of the two processes is related to increased stomatal resistance as a result of stomatal closure.

Conclusion

B. angustifolia - Z. chalybeum combination and single plant treatmentof *M. volkensii* had positive effects on enhancing the rate of photosynthesis in bean plants. The high regressions between stomatal conductance and rate of transpiration in the treatments indicated that stomatal conductance and rate of transpiration were inter-dependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. These plant extracts however caused an increase in the rate of transpiration of the bean plants, which resulted in loss of water.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Short Communication

Fatty acid composition of the seed oil of *Chrysophyllum albidum* (G.Don)

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The fatty acid composition of seed oil of *Chrysophyllum albidum* was determined by gas chromatography-mass spectroscopy (GC-MS). The seed oil contained much important fatty acids with linoleic (26.21%) and palmitic (14.41%) acids being the most abundant unsaturated and saturated fatty acids, respectively. The total unsaturation for the oil was 67.61%. These results confirm that the oil of *C. albidum* is of industrial importance.

Key words: *Chrysophyllum albidum*, fatty acid, seed oil, methyl esters

INTRODUCTION

African star apple (*Chrysophyllum albidum* G. Don) is a tropical edible fruit tree. It belongs to the family of Sapotaceae which has up to 800 species and make up almost half of the order (Ehiagbonare et al., 2008). It is primarily a forest tree species and its natural occurrences have been reported in diverse ecozones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire (Bada, 1997). The plant often grows to a height of 36.5 m though it may be smaller (Bada, 1997). Preliminary studies indicated that the oil was non-drying, based on its iodine value (Table 1) (Osamudiamen and Lukman, 2012). This paper is a report on the fatty acid constituents of the oil.

MATERIALS AND METHODS

Sample preparation

The oil was extracted using soxhlet extractor with n-hexane for 8 h and the fatty acid profile of the seed oil was obtained as fatty acid methyl esters. This was prepared by using commercial aqueous HCl as described by Ichihara et al. (2010). The reagent was made from 9.7 ml commercial concentrated HCl (35%w/w) diluted with 41.5 ml of methanol and 0.30 ml of the reagent solution were added in this order. The tube was vortexed and then heated at 100°C for 1 h. After cooling, 1 ml of hexane and 1 ml of water were added for extraction of methyl esters in the hexane phase. This was then analyzed with gas chromatography-mass spectrophotometer.

RESULTS AND DISCUSSION

The fatty acid composition of the seed oil investigated revealed that the fatty acid range was from 12:0 to 20:1 (Table 2). The saturated fatty acids comprised of lauric acid (12:0), palmitic acid (16:0) and stearic acid (18:0) while the unsaturated fatty acid comprised of two monoenes namely: vaccenic acid (18:1) and eicosenoic acid (20:1), one diene: linoleic acid (18:2) and one triene: α-linolenic acid (18:3). Linoleic (25.44%) and linolenic acid (26.12%) are the predominant unsaturated fatty acid. Linoleic acid undoubtedly is one of the most

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Table 1. Physicochemical characteristics of *C. albidum* oil.

Parameter	*C. albidum* oil
Colour	Dark yellow
State at room temperature	Liquid
Specific gravity (25°C)	0.89±0.12
Refractive index (25°C)	1.66±0.04
Mean molecular mass (g)	1332.54±0.70
Acid value (mgKOH/g)	3.56±0.59
Free fatty acid (%)	1.80±0.21
Saponification value (mgKOH/g)	126.30±0.70
Iodine value (mg iodine/g)	31.06±0.70
Peroxide value (MgO_2/g oil)	1.76±0.50

Table 2. Fatty acid composition of *C. albidum*.

Systematic name	Lipid number	Trivial name	Percentage
Dodecanoic acid	12:0	Lauric acid	0.46
n-hexadecanoic acid	16:0	Palmitic acid	14.41
Octadecanoic acid	18:0	Stearic acid	4.38
Trans-11-octadecanoic acid	18:1	Vaccenic acid	14.73
9,12-octadecandedienoic acid(z,z)	18:2	Linoleic acid	25.44
9,12,15-octadecantrinoic acid	18.3	α-Linolenic acid	26.12
Cis-13-eicoseneoic acid	20:1	Paullinic acid	1.32
Unsaturated acids			67.61
Saturated acids			19.25
Unidentifiables			13.14
Total acid			100.00

important polyunsaturated fatty acids in human food because of its prevention of distinct heart vascular diseases (Omode et al., 1995). It is well known that dietary fat rich in linoleic acid, apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, also prevents high blood pressure (Vles and Gottenbos, 1989). The presence of one of the three essential fatty acids in the seed oils make them nutritionally valuable. Palmitic (14.41%) and stearic acid (4.38%) are also present in high proportion in the oil, which are of nutritional significance

Conclusion

This study has shown that the fatty acid composition of *Chrysophyllum albidum* was predominantly linoleic, linolenic and palmitic acids. The fatty acid profile suggests the possible application of the seed and its oil as a potential industrial resource.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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An ethnobotanical study of medicinal plants in Debre Libanos Wereda, Central Ethiopia

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An ethnobotanical study of medicinal plants in Debre Libanos Wereda, in central Ethiopia, was carried out from October 2008 to June 2009. A total of 60 informants were interviewed that include knowledgeable farmers, monks, nuns, herbalist farmers and full time herbalists. A total of 83 medicinal plants classified under 77 genera and 46 families were collected. Asteraceae were the most prominent family (7) species and (6) genera, followed by Fabaceae and Lamiaceae that contain four species in three genera each. These plant species were found to be used in treating 50 different types of human and livestock diseases. The majority (77.1%) were wild species whereas 22.9% of the reported medicinal plant species were cultivated in home gardens. Higher numbers of species (46.6%) were harvested for their leaves, followed by roots, seeds and fruit (14.56, 13.59 and 6.80%, respectively). Vast knowledge on the traditional uses of these plants is conveyed from one generation to the next generation through words of mouth. As a result, there is a need for urgent biodiversity conservation of the area and the indigenous traditional ethnobotanical knowledge.

Key words: Ethnobotany, medicinal plant, herbalist, disease, mode of preparation.

INTRODUCTION

Ethnobotany is the study of the relationships between plants and people with a particular emphasis on tradetional cultures. The traditional use of plants to fulfill daily needs dates back to the beginning of human civilization and continues to date. Still traditional medicinal plant knowledge is the integral part of culture of many Asian and African countries indigenous community (Subramanyam et al., 2008; Bekalo et al., 2009). In Ethiopia, utilization of medicinal plant remedies in preventing or curing various ailments still plays a significant role in most parts of the country (Birhan et al., 2011; Giday and Teklehaymanot, 2013; Tolossa et al., 2013). Particularly, traditional herbal healing is widely practiced throughout the rural population as their primary healthcare system (Yineger and Yewhalaw, 2007; Seid and Tsegay, 2011).

There is a high expectation of enormous traditional knowledge and use of medicinal plant species in Ethiopia due to the existence of diverse languages, cultures, beliefs and significant geographical diversity which

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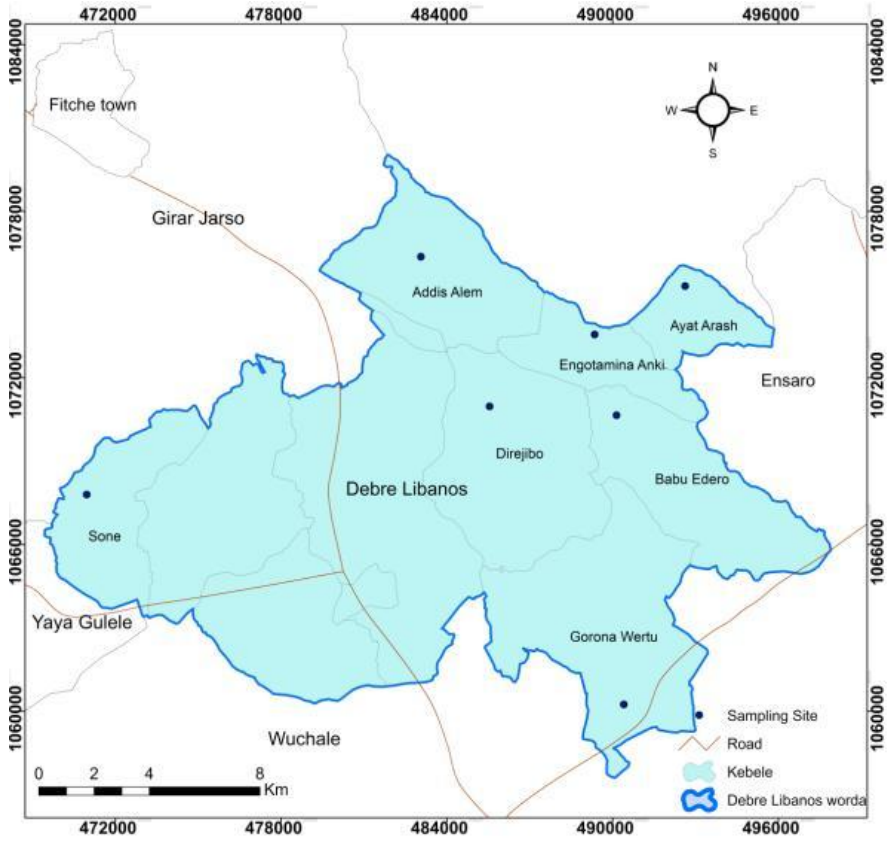

Figure 1. Location map of the study area. Data Source: Ethio-gis collected by ECSA (Ethiopian Central Statistics Authority, 2008). Software ArcGIS 9.2 was also used.

favored the formation of different habitat for medicinal plant (Cunningham et al., 2001). In Ethiopia, it has been estimated that traditional remedies are the most important and sometimes the only source of therapeutics for nearly 80% of the population of which 95% of traditional medicinal preparations are of plant origin (Hamilton, 2003). Much of the knowledge on traditional medicine is available in rural communities. Most of them are perpetuated by word of mouth within family or small community. However, since cultural systems are highly dynamic, these skills are likely to be lost when the communities emigrate to towns or regions, or if the local ecology is significantly changed (Suleman and Alemu, 2012). Furthermore, the high population pressure and its related consequences like increased need for agricultural land, settlement, fuel wood, house construction, and income generation have led to an extreme reduction of medicinal plant in all over their ranges (Bekalo et al., 2009; Belayneh et al., 2012). Teklehaymanot et al. (2006), attempted to study the medicinal plants of the present study area. But, the study covered small area as compared with the total coverage of Dere Libanos Wereda. In addition to this, the study was not able to determine the knowledge difference between the villagers and the Monastery dwellers. From this very fact, the present study on the use and management of traditional medicinal plants by indigenous people in Debre Libanos Wereda has been initiated to complete the remaining task or to fill the research gap indicated by Teklehaymanot et al. (2006).

MATERIALS AND METHODS

The study area

The study area is located in central part of Ethiopia at about 104 km north of the capital city and situated between 38° 05' 01'' to 38° 05' 51'' E longitude and 90° 40' 11'' to 90° 40' 51'' N latitude (Figure 1). The total area coverage of the study area is 29,776 hectares. The study area encompasses ten peasant associations (rural administrative divisions) and one Administrative town. Interviews were carried out in seven peasant associations (sampling sites) (Figure 1). The total population of the study area is 62,830, of this about 90.4%, that is, 56,798 people live in rural area. The altitudinal range of the study area is between 1500 and 2700 m.a.s.l (Kemal et al., 1996).

The area is characterized by bimodal rainfall during the long rainy season (June to September) and shorter rainy season (March to April). The highest average monthly rainfall was recorded in July (353.99 mm) and the lowest in November (5.5 mm). The daily average maximum temperature was recorded in the month August (17.67°C) and daily average minimum temperature 6.14°C

(Kemal et al., 1996).

The most frequent soil type in the area is blackish and red soil. Black soil being dominant of all constitutes about 56% (16,675 hectare), while the red soil comprises 38% (11,341 ha) and the rest 6% (1,787 hectare) are mixture of different soil type. The soil texture is 63% clay, 27% silt and 10% sand (Kemal et al., 1996). The vegetation of the study area is characterized by, Acacia-Commiphora dominate at lower elevations, old remnant afro montane forest in the middle altitudes, and grasslands dominant at higher elevations in the highlands. The old afromontane forest in the middle altitude is owned by Debre Libanos Monastery of Ethiopian Oriental Orthodox Church. The common vegetations in the study area are mostly remnants of trees in agricultural fields, bushes, shrubs and secondary forests. The common plant species of the study area include *Allophylus abyssinicus* (Hochst.) Radlk, *Acacia abyssinica* Benth., *Prunus africana* (Hook.f.) Kalkman, *Juniperus procera* Hochst. ex Endl. and *Olea europaea* subsp. *cuspidata* (Wall. & G.Don) Cif.

Methods

Ethnobotanical data were collected from November 2008 to January 2009. Data were collected through observation, site and informant selection, semi structured interviews, guided field walks with informants, and group discussions to obtain indigenous knowledge of the local community on medicinal plants. Local people were involved during the whole period of investigation as a guide, informants, and as technical assistances. Seven informants were asked to rank five medicinal plants that are used to treat ascariasis. Seven informants were selected from local people for pair wise comparison of medicinal plants used to treat wound.

Two types of interviews were carried out; semi structured interviews and focused group discussion. A total of 60 (50 males and 10 females) informants that include knowledgeable farmers, monks, nuns, herbalist farmers and full time herbalists, were selected. The informants were grouped into three age groups, young (27-35), adult (36-50) and elderly (above 50) to see how the knowledge varies with age. Among them 15 key informants were selected based on the information obtained from knowledgeable elders and local authorities (Development Agents workers and Peasant associations administration leaders). The other 45 informants were selected randomly from the local people of the study area to record general knowledge about plant use. This was done by tossing a coin and using him/her as informant whenever head of the coin face up if he/she volunteered to participate.

Semi-structural interviews were conducted in a place where the informants were most comfortable and during the time they have chosen. Discussion was made with volunteer traditional healers and knowledgeable farmers on the knowledge and use of important medicinal plants. At the time of discussion all informants were allowed to talk freely without interruption.

Plant identification was carried out by voucher specimens collection. Preliminary identification of the collected specimens was made in the field, and then they were dried, deep-frozen and identified in the National Herbarium (ETH), housed in Addis Ababa University using the published volumes of the Flora of Ethiopia and Eritrea and by comparing with authentic herbarium specimens and finally confirmed by assistance of taxonomists.

Data analysis

Data was analyzed and summarized using descriptive statistics such as percentage and frequency. Filter facility was employed to identify the most common ailments in the study area. In addition to this preference ranking, paired comparisons were made for some selected medicinal plants based on methods given by Martin (1995) and Cotton (1996). In the preference ranking exercise, an integer value (1, 2, 3, 4 and 5) was given, where the most important medicinal plant was given the highest value (5), while the least important is assigned a value of "1". These numbers were summed for all respondents, giving overall ranks to the medicinal plants. Informant consensus factor was also computed to evaluate the reliability of information recorded during the interview using the formula below (Martin, 1995):

$$ICF = \frac{n_{ur} - n_i}{n_{ur} - 1}$$

Where n_{ur} is citations in each category, n_i = number of species used.

RESULTS

A total of 83 medicinal plant species, distributed across 46 families and 77 genera, have been reported to be utilized by the local people in Debre Libanos Wereda as a remedies against various human and livestock ailments (Table 1). Asteraceae appeared as the most prominent family that contains 7 species, within 6 genera, followed by Lamiaceae and Fabaceae (4 species, 3 species). 83 medicinal plant species reported by local peoples in the study area as remedies for human and livestock diseases, 77 species (92.7%) are reported as a remedy for human ailments and 6 species (7.3%) for livestock.

Herbs were the dominant life forms among the reported medicinal plants that contained 32 species (38.6%) followed by shrubs with 30 species (36.1%). Trees (15 species, 18.1) and climbers (6 species, 7.2) were least abundant life forms of medicinal plants recorded from the area. Among the medicinal plant species recorded in the study area, the majority 64 species (77.1%) were wild, whereas 19 species (22.9 %) were cultivated in home gardens.

Plant parts widely used by local people in the study area to treat human and livestock diseases include leaves, root, seed, and fruits. The maximum percentage of the species (46.6%) was harvested for their leaves followed by roots, seed and fruit (14.56, 13.59 and 6.80%) respectively as a source of remedies. Other parts consist of only 18.45% (Table 1).

A significant percentage (54.3%) of the medicinal plant was used in fresh form for remedy preparations. Relatively few medicinal plants (34.9 %) were reported to be used in dried form and the remaining very few medicinal plants were reported to be used either fresh or dried (10.8%). Very common methods of remedy preparation in the study area were reported to be through crushing or pounding the usable part by using wooden or stone made material that cover 50.64% followed by squeezing (12.84%), chewing (11.0%), decoctions (7.34%), covering (6.42%), chopping (3.67%) and smoke (1.84%) (Figure 2). Local peoples also used additives such as butter, edible oil for wound and skin disease, and they used coffee, honey, and local beverages like

Table 1. Plant species used for treatment of human and livestock diseases and their mode of preparation and application in Debre Libanos Wereda, Ethiopia.

S/N	Botanical name	Family name	Local name	Disease treated	Mode of preparation and application
1	*Acacia abyssinica* Hochst. ex Benth.	Fabaceae	Girar	Goat intestinal parasite	Crush the seed together with fruit and make a juice by mixing with cold water; give to the infected animal as a drink
				Nose bleeding	Squeeze flashy leave and drop to nostrils.
2	*Achyranthes aspera* L.	Amaranthaceae	Tilenj	Anthrax	Crush the leaves and mix with fruit of *Cucumis ficifolium* and make a juice with cold water then give the cattle as a drink.
3	*Allium cepa* L.	Alliaceae	Keye shinkurt	Eye	Squeeze the leave and drop the fluid in to the eye.
				Asma	Squeeze the bulb and take one tea spoon every morning
				malaria	Chew the bulb and swallow it
4	*Allium sativum* L.	Alliaceae	Nech shinkurt	Common cold	Crush the bulb and swallow it. Additionally, insert the bulb in the nostrils.
				Snake bit	Crush the bulb and put it on the site of bites and tie it
				Abdominal pain	Crush the bulb and mix with honey take a tea spoon each morning.
5	*Allophylus abyssinicus* (Hochst.) Radlkofer	Sapindaceae	Enbis	Skin itching	Squeeze the leave and rub on the skin
6	*Amaranthus caudatus* L.	Amaranthaceae	Bahr tef	Intestinal disorder	Crush the seed and mix with black teff flour then make a bread to eat.
				Diarrhea	Squeeze the leaves and make a juice
7	*Artemisia rehan* L.	Asteraceae	Arity	Diarrhea	Boil leaves with water and dink half cup of coffee the hot decoction every day
8	*Asparagus africanus* Lam.	Asparagaceae	Serity	Abdominal pain.	Fresh root of this plant is chewed and swallowed
				Tooth ache	Chew the root and hold it near the infected teeth.
9	*Bersama abyssinica* Fresen.	Melianthaceae	Azamir	Ascaris	Fresh leaves are boiled in water and drunk for three consecutive days.
10	*Brassica oleracea* L.	Brassicaceae	Yabesha gomen	(Stomack burn	Boil the leaves and mixed with oil of *Nigela sativa* L. and eat with injera.
11	*Brusea antidysenterica* J.F.Mill.	Simaroubaceae	Abalo	Wound and Skin itching	Crush leaves and mix with butter then cover the wound
12	*Buddleja polystachya* Fresen.	Loganiaceae	Anfar	Eye disease	Squeeze the leave and drop on the infected eye
13	*Calotropis procera* (Ait.) Ait.f.	Asclepiadaceae	Kobo	Wound	Squeeze fresh leave and pour the content on wound, or put the leave on fire then cover the wound with fired leave.
14	*Calpurnia aurea* (Alt.) Benth.	Fabaceae	Digita	Abdominal pain	Crush the leave and mix with coffee powder then make a juice with water.
				External parasite	Chop the leave and put it in water for few days then wash the affected site.

Table 1. Contd.

15	*Capparis tomentosa* Lam.	Capparidaceae	Gumero	Spiritual disorder	Crush the root and put it on fire and smoke the bedroom of the patient
				For any sudden ailment	Crush the root and mix with Tela then a glass of the mixture is given as a drink.
16	*Carica papaya* L.	Caricaceae	Papaya	Amoeba	Crush seeds and mix with honey and water then drink the juice each morning for a week.
17	*Carissa spinarum* L.	Apocynaceae	Agam	Spiritual disorder (unable to sleep at night)	Make a powder from the root and mix with water, put a spoon full in to a cup of coffee and drink
				Evil eye	Smoke the root all over the body of the patient
18	*Catha edulis* (Vahal.) Forssk. ex Endl.	Celastraceae	Chat	Coughing	Boil the leave and stem with water then add honey and then set it aside to get cooled. At last drink a glass of cold mixture.
19	*Citrus limon* (L.) Burm.f.	Rutaceae	Lomi	Asma	Boil the leave of *Citrus limon* together with stem of sugarcane then drink hot decoction.
20	*Clematis simensis* Perr and Guill	Ranunculaceae	Yeazo hareg	Wound	Leaf of *Clematis simensis* are crushed, smashed and tied on wound.
21	*Clerodendrum myricoides* (Hochst.) R.Br.ex Vatke.	Verbenaceae	Misireg	Spider poison	Crush leaves and mix with butter then rub on the affected skin.
22	*Cordia africana* Lam.	Boraginaceae	Wanza	Gastric ulcer	The seed is Chewed and swallowed
23	*Croton marcostachyus* Del.	Euphorbiaceae	Bisana	Skin disease	The leaves are squeezed and the content is dropped on the infected site.
24	*Cucumis ficifolium* A.Rich.	Cucurbitaceae	Yemdir enboay	Wound	Roots are Crushed and mixed with butter are put on wound.
				Snake bite	Chewing the root and swallowing the juice only
25	*Cucurbita pepo* L.	Cucurbitace ae	Duba	Tape worm	Roost the seed and eat
26	*Datura stramonium* L.	Solanaceae	Astenagir	Dandruff	Crush leaves and seed of this plant mix with butter then apply the paste to affected area (head).
				Wound	Leaves are crushed and applied to affected area.
27	*Diplolophuium africanum (*Turez*.)*	Apiaceae	Feres zeng	Ascariasis	Squeeze leaves, mix with water and drink (for children)
28	*Dodonaea angustifolia* L.f.	Sapindaceae	Kitkita	Wound	Leaves are crushed and applied on wound
29	*Dorstenia barnimiana* Schweinf.	Moraceae	Work bemeda	Leprosy	Crush the root, mix with seeds of *Lepidium sativum* L. and sorghum flour and then extract local alcohol (Areke) from it, then the patient drinks it until he/she recovers.
				Hypertension	Root and stem tuber are smashed, mixed with ‘Tela’ and drink
30	*Dovyalis abyssinica* (A.Rich.) Warb.	Flacortiaceae	Yabesha	Acariasis	Boil seeds with water and drink.
			Qoshm	Bleeding gum	Eat fresh fruit
31	*Ekebergia capensis* Sparmm.	Meliaceae	Sembo	Skin rush	Crush leaves and mix with butter and apply on the site of infection.

Table 1. Contd.

32	*Eucalyptus globulus* Labil	Myrtaceae	Nech bahirzaf	Sudden physiological change	Boil leaves in water and inhale the vapor
33	*Euphorbia abyssinica* J.F.Gmel.	Euphorbiaceae	Qulqual	External parasite Wound	The latex (milky juice) is applied to affected area. Milky juice(latex) is applied on wound
34	*Ferula communis* L.	Apiaceae	Inslal	Hypertension Unable to urinate	Crush leaves add to boiled tea and drink. Crush leaves mix with water and drink.
35	*Ficus vasta* Forssk.	Moraceae	Warka	Constant lose of weight in cattle	Chop the bark boil it in water pour to the moth of the cattle
36	*Ficus palmata* Forssk.	Moraceae	Beles	Skin rush	Crushed leaves are mixed with butter then the paste is applied on the affected site.
37	*Glinus lotoides* L.	Aizoaceae	Metere	Tooth ache Tape worm	Chew the root and hold tightly near the infected teeth. Crush seed and mix with Lin seed and then eat it.
38	*Glycine wightii* (Wight &Arn.) Verdc.	Fabaceae	Yelam chew	Constant lose of weight in cattle)	Crush fresh seed and leaves make a juice like fluid pour to the moth of the cattle.
39	*Grewia flavescens* Juss.	Tiliaceae	Lenquata	Bloating	Crush leaves mix with water and drink.
40	*Helinus mystacinus* (Ait.) E. Mey.ex Steude.	Rhamnaceae	Shnbirit	'Globa' (Cattle disease)	Leaves and stem are Crushed and boiled in water and put aside until it gets cooled. Then pour in to the mouth of the cattle.
41	*Jasminum floribundum* R.Br	Oleaceae	Tembele	Eye treatment Tape worm	Squeeze leave and put dropsthrough ear. Leaves are crushed, mixed with water and then the patient will take a glass of the mixture as a drink.
42	*Laggera tomentosa* (Sch. Bip.ex A. Rich.) Oliv. & Hiern	Asteraceae	Nech kese	Common cold	Hold fresh leaves tightly in to the nostrils
43	*Leonotis ocymifolia* (Burm.f.) Iwarsson	Lamiaceae	Eras kimir	Abdominal pain	Squeeze leaves add in a cup of coffee and drink.
44	*Lepidium sativum* L.	Brassicaceae	Feto	Abdominal pain	Crush seed and mix with other dishes ('Injera')
45	*Linum usitatissimum* L.	Linaceae	Telba	Intestinal wound	Crush the seeds in to powder and mix with water then drink a glass of juice before food each morning until recovery
46	*Lycopersicon esculentum* Mill.	Solanaceae	Timatim	Weak feeling	Squeeze the fruit and mix with Allium cepa L. Mix the juice in a cup of tea and drink it every morning
47	*Maesa lanceolata* Forssk.	Myrsinaceae	Kelawa	Skin disease Characterized by itching and black spots	Crush seed and mix with butter then apply on infected site.
48	*Momordica foetida* Schumach. & Thonn.	Cucurbitaceae	Ababure	Swelling wound	Roots are crushed mixed with butter and applied on the affected site.

Table 1. Contd.

49	*Myrsine africana* L.	Myrsinaceae	Kechemo	Endoparasites (Tape worm and Ascaris)	Crush fruits and make a juice then drink.
50	*Myrtus communis* L.	Myrtaceae	Ades	Headache	Crush leaves and boil with water then drink with cup.
51	*Nicotiana tabacum* L.	Solanaceae	Timbaho	To remove leeches from cattle mouth	Crush dry leaves mix with water and give it to cattle as a drink.
52	*Ocimum lamiifolium* Hochst. ex Benth.	Lamiaceae	Damakese	Headache and cough	Leaves are Squeezed and drunk alone or with coffee.
53	*Osyris quadripartita*. Decn.	Santalaceae	Keret	Abdominal pain Urine problem	Chewing the stem and swallowing the fluid only. Crush and boil with water and drink.
54	*Otostegia integrifolia* Benth.	Lamiaceae	Tungut	Abdominal pain Evil eye	Crush laves mix with water and drink. Burn stem and leaves and in hale the smoke
55	*Otostegia fruticosa* (Forssk.)Schweinf.ex Penzig	Lamiaceae	Geram tungut	Tonsillitis	Squeeze leaves while adding few drops of water and drink the juice.
56	*Pentas schimperiana*	Rubiaceae	Ese zeye	Snake bite	Chewing the root and swallowing only the liquid part of root.
57	*Phytolacca dodecandra* L.Herit.	Phytolaccaceae	Indod	Abortion	Crush the root and mix with water and drink.
58	*Premna schimperi* Engl.	Verbenaceae	Chocho	Toothache Eye disease	Chew the stem and hold it tightly to the infected teeth. Squeeze fresh leaves and drop a drop of the extract on the affected eye.
59	*Prunus persica* (L.) Batsch.	Rosaceae	Kok	Appetite	Eat fruits
60	*Pterolobium stellatum* (Frossk.) Brenan	Fabaceae	Kentafa	Goiter	Crush leaves mix with butter then apply the paste and tie it and cover it
61	*Rhus retinorrhoea* A.Rich.	Anacardiaceae	Tilem	Liver infection	Roots of *Rhus retinorrhoea* crushed together with flower of *Catha edulis* and (*Rumex nervosus*) and mixed with water and add small amount salt then drink
62	*Ricinus communis* L.	Euphorbiaceae	Gulo	Eye disease	Leaves are slightly heated and applied on the affected part of the eye
63	*Rosa abyssinica* Lindley.	Rosaceae	Kega	Gastric	Eat fruit and seeds
64	*Rumex nervosus* Vahl	Polygonaceae	Enboacho	Anti-bleeding	Crush dried leaves in to powder and apply on the cut.
65	*Rumex abyssinicus* Jacq	Polygonaceae	Mekemeko	Hypertension	Crush root in to powder mix with bubs of *Allium sativum* add the mixture in to boiled water and drink the hot decoction in a cup
66	*Rumex nepalensis* Spreng.	Polygonaceae	Tult	Diarrhea	Crush root mixed with water and drink

Table 1. Contd.

67	*Ruta chalepensis* L.	Rutaceae	Tenadam	Abdominal pain	Stem and leaves are crushed and added in to a cup of tea or coffee and drunk or chewing fresh stem and leaves and swallowing
68	*Schinus molle* L.	Anacardiaceae	Kundo berbere	Tuberculosis	Crushed seeds are mixed with honey and then eaten.
69	*Sesamum angustifolium* (Oliv.) Engl.	Pedaliaceae	Selit	Ear defect	Extract oil from the seed and drop the extract it the ear
70	*Solanecio gigas* (Vatke) C.Jeffrey.	Asteraceae	Yeshe koko gomen	Skin disease	Leaves are crushed and pasted on affected body
71	*Solanum anguivi* Lam.	Solanaceae	Dekak enboay	Skin disease (itching)	Dry seeds are crushed, mixed with oil and applied on the site. For fresh seeds no need of using oil.
72	*Steganotaenia araliaceae* Hochst.	Apiaceae	yegibmrkuz	To remove leaches from cattle	Crush leaves boil with water then pour in to the mouth of the cattle
73	*Stephania abyssinica* (Dillon. et A.Rich.) Walp.	Menispermiaceae	Yayit hareg	Wound	Roots are Crushed and mixed with milk and applied on wound.
74	*Trigonella foenum-graecum* L.	Fabaceae	Abish	Abdominal pain	Crush seeds and make a juice by mixing it with water then add hone to drink
75	*Verbascum sinaiticum* Benth	Scrophulariaceae	Yahiya joro	Skin disease	Leaves are crushed and mixed with water wash the affected part with the mixture and tie with the soaked leave.
76	*Verbena officinalis* L.	Verbenaceae	Atuch	Abdominal pain	Dry roots are crushed and mixed with water and drunk. For fresh root, chew and swallow only the liquid part of root.
77	*Vernonia amygdalina* Del.	Asteraceae	Girawa	Abdominal pain	Crush leaves in to powder and mix with water then drink.
78	*Vernonia bipontini* Vatke	Asteraceae	Gobez tekes	Toothache Eye disease	Chew leaves and hold it close to the infected teeth. Squeeze leaves and drop one or two drops of the extract on the eye
79	*Withania somnifera* (L.) Dunal	Solanaceae	Gizaw	Spiritual disease.	Smoke the entire body of the patient with dried leaves
80	*Xanthium strumarium* L.	Asteraceae	derkus	Skin rush	Leaves are crushed and mixed with butter and applied on the infected site.
81	*Ximenia caffra* Sond.	Olacaceae	Atat	Snake bite	Take seven leaves of this plant and chew it well then swallow the juice
82	*Zehneria scabra* (Linn.f.)Sond	Asteraceae	Hareg ressa	Wound	Leaves are crushed and mixed with oily substance and applied on the infected site.
83	*Zingiber officinale* Rosc.	Zingiberaceae	Zingible	Abdominal pain	Direct eating or crushed and mixed with tea and drunk.

'Tela' and 'Areke' for those plants having bitter taste.

The most common mode of administration in the study area was oral that cover 49.4%. Most of the remedies prescribed by traditional healers are applied in various ways such as drinking like a

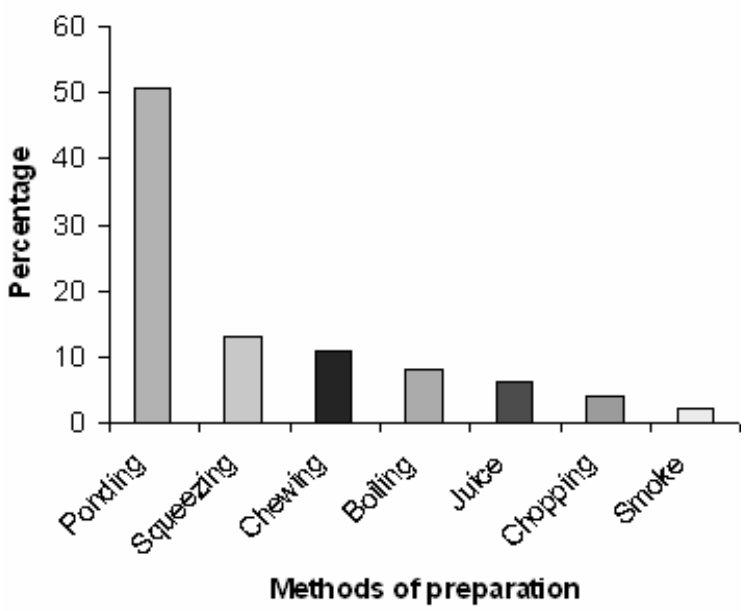

Figure 2. Mode of preparation of medicinal plants.

Table 2. Informant consensus factor of medicinal plants of Debre Libanos Wereda, Ethiopia by categories of diseases.

Categories (diseases)	No of species	Percentage of total species	No. of informant	Percentage of no. of informant	ICF (%)
Skin diseases and wounds	21	25.3	45	19.7	54.5
Respiratory infections and cough	5	6	11	4.8	60
Intestinal parasites, abdominal pain, diarrhea.	14	16.9	52	22.8	74.5
Malaria and hypertension	4	4.8	8	3.5	57.1
Snake bites and spider poisons	5	6	9	4	50
Common cold, headache and sudden physiological change	7	8.4	55	24	88.9
Spiritual disorder	5	6	6	2.6	20
Swelling and bleeding	7	8.4	9	4	25
Organs disease (eye, teeth, kidney, liver)	10	12	18	7.9	47.1
Cattle disease (bloating anthrax, leaches and external parasites)	5	6	15	6.6	71.4

juice, take a drop of squeezed plant part or chewing and swallow the liquid part only, etc. Dermal is the second most important route of administration of traditional medicine which covers 33.7%. There are various ways of dermal application oftraditional medicine. For example, they may apply as a form of paste, coated and tie or crushed the plant part and put the powder on the affected part and so on. Only few medicinal plants were reported to be administered through oral and nasal, eye, nose and ear (about 16.9%).

Almost all traditional healers in the study area do not have sufficient knowledge on dosages. An ethnobotanical data in this study showed that all administrations are not standardized. Healers determine the dosages based on age, physical appearance and strength of the disease. Children are given small doses of medicine than considered in case of adult patient. Small amount such as drops, hand palms, coffee cups are applied as small dosage. For larger dosages, they use water glasses or other local materials that are used for drinking.

The highest percent informant consensus factor (% ICF) value was obtained with problems associated with common cold, headache and sudden physiological change (febrile illness characterized by fever, headache, skin rash and muscle spasm) (88.9%) followed by problems related to intestinal parasites, abdominal pain, diarrhea (74.5%). The lowest %ICF value was obtained in diseases associated with spiritual disorder (20%) (Table 2).

Table 3. Informant consensus.

Botanical name of medicinal plant	No. of informants	Percentage
Glinus lotoides	60	100
Ocimum lamiifolium	60	100
Ruta chalepensis	60	100
Zingiber officinale	60	100
Allium sativum	55	91.7
Croton macrostachyus	50	83
Brucea antidysenterica	49	81.7
Vernonia amygdalina	45	75
Lepidium sativum	38	63
Cucumis ficifolius	35	58

Table 4. Preference ranking of medicinal plants of Debre Libanos Wereda, Ethiopia used to treat Ascariasis.

Lists of medicinal plants	Informant labeled from R1-R7								
	R1	R2	R3	R4	R5	R6	R7	Total	Rank
Ruta chalepensis L.	3	4	4	3	3	4	3	24	3
Malva verticillata L.	5	5	4	4	5	5	5	33	1
Diplolophium africanum Turcz.	4	3	3	2	1	2	3	18	5
Vernonia amygdalina Delile	3	3	4	3	3	3	2	21	4
Lepidium sativum L.		4 5 5		5	4	4	4	31	2

The study revealed that some medicinal plants are well known for their medicinal value among the herbalist and the local community at large than the other. As a result, local informants cited such plants repeatedly as a remedy of different diseases. For example, *Glinus lotoides* L., *Ocimum lamiifolium* Hochst. ex Benth and *Ruta chalepensis* L. were cited by 60 informants (100%) as a source of remedy for tapeworm, sudden physiological change, and abdominal pain respectively (Table 3). *Allium sativum* L. was cited by 55 informants (91.7%) as a remedy for malaria, common cold and other illness while *Croton macrostachyus* Hochst. ex Delile was cited by 50 informants (83%) for gastric ulcer. On the other hand, *Lepidium sativum* L. and *Cucumis ficifolius* A.Rich. were cited as the least commonly used medicinal plants by the informants (58%) (Table 3).

Preference ranking was made for five most important medicinal plants that are used to treat abdominal problems associated with ascariasis. Among the five selected medicinal plants *Malva verticillata* L. stands first followed by *L. sativum* L. (Table 4).

The result of pair wise comparison indicates that *Brucea antidysenterica* J.F.Mill. followed by *Calotropis procera* (Aiton) Dryand. and *Stephania abyssinica* (Quart.-Dill. & A.Rich.) Walp. ranked first, second and third, respectively for the treatment of wound. The other two less preferred medicinal plants for wound treatment were *Clematis simensis* Fresen. and *Verbascum sinaiticum* Benth. (Table 5).

DISCUSSION

The number of reported medicinal plants (83 species, 77 genera and 46 families) and their uses by the community witness show how rich the area is in terms of medicinal plants diversity and the depth of the local indigenous knowledge on medicinal plants and their applications. A number of studies elsewhere in Ethiopia have reported similar number of species of medicinal plants as traditional medicines against human and livestock alignments (Yinger and Yehwalahu, 2007; Bekalo, 2009; Tolosa et al., 2013). The relatively higher number of traditional medicinal plant species documented from the study area is mainly attributed to strict conservation of forest resources of the monastery. The monastery harbors various medicinal plants including those medicinal plants that have been widely used long time ago. The monastery has played a key role in conserving the remnant afro montane forest which is a source of most medicinal plant species, even though any plant in the monastery is strictly forbidden to be used as traditional medicine. Furthermore, the acceptance of folk medicine and the limited access to public healthcare services in the community may be factors contributing to the knowledge of medicinal species in local medical practices. However, the traditional systems and religious beliefs that generally restrict the way of transferring indigenous knowledge might have constrained, to some extent, the free flow of information on medicinal plants in

Table 5. Pair wise comparison of medicinal plant of Debre Libanos Wereda, Ethiopia used to treat wound.

Medicinal plant	Informant labeled from R1-R7								
	R1	R2	R3	R4	R5	R6	R7	Total	Rank
Brucea antidysenterica J.F.Mill.	4	5	4	5	5	4	5	32	1
Calotropis procera (Aiton) Dryand.	4	5	5	5	4	4	3	30	2
Clematis simensis Fresen.	3	4	2	3	5	3	4	24	4
Stephania abyssinica (Quart.-Dill. & A.Rich.)	4	3	5	4	3	4	5	28	3
Verbascum sinaiticum Benth.	4	3	4	2	3	4	2	22	5

this study. Secondly, it could be attributed to the scope of the study that attempted to study the medicinal plants in there agro ecological zones, lowland, middle elevations and highlands unlike Teklehaymanto et al. (2006) that confined his study only in the monastery and its immediate vicinity.

The trees and shrubs constitute more than 70% of the traditional medicinal plant in the study area. This could be attributed to various factors. First it can be related with the floristic composition of the vegetation of the area, which is dominated by herbs and exotic *Eucalyptus* sp. in the high lands and Acacia-commiphora woodland in the low lands. Secondly, a high usage of herbs could be an indication of their abundance, since the area receives relatively high amount of rain fall that fosters the flourishing of herbs. Thirdly, the affinity to use herbs as traditional medicinal plant could be attributed to strong bioactive compounds. Studies in various parts of the world have raveled that herbs contain phytochemicals like alkaloids and falvanoids that have strong anti bacterial and anti fungal effects (Legesse et al., 2011). A number of studies carried out elsewhere in Ethiopia have documented that herbs and shrubs are plant species mostly used by indigenous communities of Ethiopia as treatment against various human and livestock ailments (Teklehymanot et al., 2006; Bekalo et al., 2009; Mesfine et al., 2009) . For example, Konta people use more of herbs (about 68 species) than trees (20 species) (Bekalo et al., 2009) in a similar pattern as reported from India, where about 19 out of 54 species were herbs and shrubs were about 12 species (Ayyanar and Ignacimuthu, 2005). More than half of the Zay plant remedies were also obtained from herbs (Giday et al., 2003)

Most of medicinal plants (75%) utilized by local people of the study area are collected from wild; only few (25%) are harvested from home garden. There is little tradition or practice by local people to cultivate medicinal plants. Plants were harvested and processed only when needs aroused. The use of uncultivated plants is a common practice in Ethiopia (Giday and Ameni, 2003) and this has been creating an additional pressure on the populations of wild plants besides enviromental degradation and deforestation. For example, similarly Gebre (2005) found that about 76.5% of the remedies were reported from wild. The same author also noted that most of the medicinal plants are under the threat as long as the destruction and fragmentation of wild habitat continues. This is also true in the present study area. Furthermore, Asfaw and Woldu (1997) reported that only 6% of the plants maintained in home gardens in Ethiopia are primarily cultivated for their medicinal value even though many other plants grown for non-medicinal purposes are used for preparation of medicines.

In the present study, the leaf is one of the most extensively used plant parts in preparation of traditional herbal medicine followed by root and seed. The common use of leaf in the preparation of remedies could partly be due to the relative ease of finding this plant part. The practice of using leave part for remedies preparation helps to reduce the rate of threat on plant species or helps for sustainable harvesting of plants since removal of an appreciable amount of leaf is tolerated by the plant. Roots appeared also to be the second most plant part commonly used by the healers in the current study area. This could be associated with the fact that roots remain in the soil and are easily available, even during the long dry seasons in arid and semi-arid areas. But, harvesting roots for medicinal value could possibly put a strain on the survival of the plant since aerial parts of the plant are highly dependent on underground parts (root) for physical support and physiological process. In agreement with our study, similar studies in other parts of Ethiopia reported that roots and leaves are indeed the most commonly used medicinal plant parts (Bekalo et al., 2009; Bussmann et al., 2011). Inspection of the results on number of preparations and plant parts used may lead to the conclusion that harvesting medicinal plants for use in traditional medicine is not destructive to the natural vegetation of the study area since leaves are the most frequently sought parts of the plant. On the other hand, it may also lead to the conclusion that harvesting of medicinal plants is likely to be destructive because the second most frequently used part is the root.

According to the present survey, a significant number (56.88%) of the medicinal plants were to be used in fresh form in remedy preparations. This indicates that local people of the study area are highly dependent on fresh remedies that may put medicinal plants under serious threat, since there is no habit of preservation or storage plant parts for later use. Ethnobotanical studies of medicinal plants elsewhere in Ethiopia have documented the same mode of preparation (Seifu, 2004; Gebre, 2005;

Amenu, 2007; Beyene, 2007).

Very dominate methods of remedy preparation in the study area were reported to be through crushing (grinding) followed by squeezing, and chewing. Local people also used additives such as butter, edible oil for wound and skin diseases, and cup of coffee, honey, and local beverages like 'Tela' and 'Areke' for those plants having bitter taste. This finding is consistent with some reports elsewhere in Ethiopia (Tekelhaymanot and Giday, 2007; Bekalo et al., 2009; Flatie et al., 2009) but disagrees with some reports where other methods of remedy preparation are employed (Abebe and Ayehu, 1993; Yirga 2010). It is likely that these differences are associated with the differences in culture and knowledge in different socio-cultural groups.

The choice of oral administration may be related to the use of some solvents or additives (butter, edible oil, coffee, honey and local beverages like 'Tela' and 'Areke') that are commonly believed to serve as a vehicle to transport the remedies. The additives are also important to minimize discomfort, improve the taste and reduce adverse effects such as vomiting and diarrhea, and enhance the efficacy and healing conditions (Etana, 2010). Similar findings were reported by many other researchers, indicating the oral route as the most preferred mode of administration (Filate et al., 2009; Mesfine et al., 2009; Addisie et al., 2012).

Dosage is not always well measured in most of the traditional medicine practitioners. The result of this study also showed that all administrations are not standardized. Other similar findings were reported by many other researchers (Yinger and Yehwalahu, 2007; Tolosa et al., 2013). Although, most of the remedies were reported to have no serious adverse effects except vomiting and temporary inflammations. This could be attributed to the low toxicity of the remedy preparations of the medicinal plant species used by the traditional healers in the study area. However, the toxicity of some medicinal plants and their potential to do harm is a common complaint among those who would like traditional medicine to be standardized. It is commonly believed that traditional practitioners either do not know the strength of their own medicines or do not bother to fit doses to the size or body weight of the patients (Hillenbrand, 2006). However, it is known that some traditional healers do give different dosages and frequency of application depending on age, sex and other condition or vary the medicine itself on such differences.

The highest numbers of plant species were reported to be used for treatment of intestinal parasites, abdominal pain and diarrhea indicating that there is relatively high consensus on the treatment of gastro intestinal problems with the medicinal plants of the area. This is manily attributed to the highest pevalance of gastro intestinal problems aligments prevalent in the study area. A similar analysis found high value of informant consensus factor (ICF) for gastrointestinal illness in similar studies carried out in different parts of Ethiopia (Tolosa et al., 2013). According to the information obtained from Debere Libanos local health center, intestinal parasites were the first among the top ten diseases treated in the health center in the year 2008. The frequent occurrence of these diseases might have given the chance for herbalists to develop diversified knowledge associated with this problem. The ICF results could be useful in prioritizing medicinal plants for further scientific validation of plants and plant products (Giday et al., 2006; Subramanyam et al., 2008) as pharmacologically effective remedies are expected from plants with higher ICF values (Trotter and Logan, 1986; Etuk and Mohammed, 2009). Indeed, documentation of inherently rich traditional ethno-medicinal knowledge based on ICF values have provided valuable information on new pharmacological dimensions for better health care of livestock and humans regarding many ailments (Etuk and Mohammed, 2009) and also assist conservation and management of rare, gradually vanishing important ethno-medicinal plant species

Traditional healers of the study area use different plants for the same ailment. But, when all plants are available at the same time they prefer one over the other. This is mostly done based on the effectiveness of the plants to cure the ailment. As a result, both preference ranking and paired comparison revealed *Malva verticillata* and *Brucea antidysenterica* as the most effective medicinal plants for the treatment of ascariasis and wound, respectively, at least in the context of local people. This indicates that indigenous people of the study area have sufficient knowledge on the healing power of medicinal plants.

It has been agreed that threats to biodiversity are increasing dramatically from time to time in such a way that the rate of biodiversity loss outweigh the rate of recruitments. Informants have pointed out that deforestation to expand agricultural land and for firewood collection are the major threats that threatened the survival of medicinal plants. Similar studies in different localities of Ethiopia have documented that medicinal plants are under severe anthropogenic threats. For example, wild habitats are subjected to the loss of a number of plant species due to different anthropogenic factors such as firewood collection (24.8%); frequent fire (22.3%) and harvesting medicinal plants for use in construction (19%) (Bekalo et al., 2009). A study conducted in Sekoru District (Yinger and Yehwalahu, 2007) has also showed that, there are different threats to medicinal plants such as deforestation (40%), drought (17.5%), agricultural expansion (12.5%) and fire (12.5%).

This study revealed that, most of the knowledge on herbal remedies is handled down to the younger members of the community by elders, who are 41-50 years old. This hints at the fact that ethno-medicinal knowledge is concentrated in the elderly members of the community and the relative difficulty in its transfer from the elders to the young generation. This might be related

to the waning of interest of the young generation on indigenous knowledge. Furthermore, since all most all part of the forest belongs to Debre Libanos Monastery of Ethiopian Oriental Orthodox Church, harvesting of any part of medicinal plant is strictly forbidden. A canon called "GIZIT" in the church language prohibits harvesting of any plant from the church compound. Move over, person who uses medicinal plants from the church compound or nearby area is believed not to get the expected cure from the health problem because of "GIZIT". As a result, many medicinal plants that are rare in other parts of the study area are found in the church compound or nearby area. These might appear to prevent the use of available medicinal plants by the herbalist and impart the expansion of the traditional medicine somehow. However, the strict conservation of the plant species by monastery is utmost important from the whole biodiversity conservation point of view. As a means for sustainable utilization of medicinal plants, plants in the church compounds and nearby area must be protected and used as a source of seeds for requirement and herbalist can now plant the seeds and grow the medicinal plants and use them as they wish. It might seem that herbalists are restricted to use. Different studies in different areas showed that medicinal plant knowledge and transfer of knowledge to the young generation have been affected by modernization (having access to modern education and health service) and environmental change (Bekalo, 2009; Tolosa et al., 2013). Furthermore, Western style health care services as provided by governments and NGOs, in particular in rural areas, seem to have contributed to a decline in traditional knowledge, in part because the local population simply regards Western medicine as more effective and safer.

Conclusion

The study area is home for several medicinal plants. These plants have a great value for the health problems of poor local people. Herbalists and knowledgeable farmers are using these plants to cure human and livestock diseases. The young generations have not shown much interest in this life long accumulated knowledge. This tendency of disinterestedness in traditional medicinal practices is likely to be one of the major causes for loosing this wealth of knowledge in the near future. Therefore, it is important that the government create awareness among community members on the significance of preserving traditional knowledge and conserving medicinal plants before they disappear, and thereby ensure the rights of people to apply their traditional practices which are known for their proven safety and effectiveness.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Community structure, regeneration potential and future dynamics of natural forest site in part of Nanda Devi Biosphere Reserve, Uttarakhand, India

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Realizing the overarching values of forests and considering their depletion at unprecedented rate, conservation of forests has emerged as the prime objective across the globe. Forest vegetation of Pindari-Sunderdhunga-Kafni (PSK), a protected area, part of Nanda Devi Biosphere Reserve in west Himalaya was analyzed for structure, composition and development of future compositional patterns. Forest vegetation surveys were carried out enumerating ten 10 x 10 m quadrat for tree species in each of 30 forest stand complemented by shrub (five 2 x 2 m quadrat) and herb (ten 1 x 1 m quadrat) surveys within each stand. Floristic richness reveals 332 plant species from 11 representative forest communities. Broadly, the demographic profiles exhibited progressive structures suggesting long term persistence of the communities/species. Differences in regeneration behavior of various species are indicative of future structure and dynamics of the communities. Data sets in the present study established target site in NDBR as potential sites for long-term ecological monitoring under various change scenarios.

Key words: Natural forests, regeneration pattern, population structure, compositional changes.

INTRODUCTION

Forest composition, community structure and diversity patterns are important ecological attributes significantly correlated with prevailing environmental as well as anthropogenic variables (Gairola et al., 2008; Ahmad et al., 2010). Forests are always characterized by their three main life stages called seedling (newly emerged plants), sapling (established plants stands between seedling and tree) and tree (tree undisturbed of micro environmental conditions). The number (density) and type (richness) of trees define the structure and composition of forest (Shankar, 2001; Mishra et al., 2003). Species richness patterns in relation to the environment need to be understood before drawing conclusions on the effect of biodiversity on ecosystem processes. Numerous problems regarding the study of species richness need to be clarified, including the role of disturbance (Huston,

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1994), and the relative importance of biotic versus abiotic factors (Cornell and Lawton, 1992; Austin and Gaywood, 1994). The number of tree individuals at seedling, sapling and tree level reveals population structure and their establishment at seedling and sapling level represent regeneration status (Baduni and Sharma, 2001; Bhandari, 2003). The nature of forest communities depends on the ecological characteristics in sites, species diversity and regeneration status of species (Criddle et al., 2003; Todaria et al., 2010). The tree species strata, that is, seedling, sapling and tree layers of the plant communities that maintain the population structure of forest get affected by micro-environmental factors which vary with seasonal changes (Khumbongmayum et al., 2006; Kharkwal et al., 2009). Environmental variation within a small geographical area makes altitudinal gradients ideal for investigating several ecological and biogeographical hypotheses (Korner, 1998). Hence, it becomes necessary to understand the species richness, population structure, germination and establishment of seedlings and saplings across seasons and altitude for maintenance of forest (Khumbongmayum et al., 2006; Rao, 1988). Complete absence of seedlings and saplings of tree species in a forest indicates poor regeneration, while presence of sufficient number of young individuals in a given species population indicates successful regeneration (Saxena and Singh, 1984). Presence of sufficient number of seedlings, saplings and young trees is greatly influenced by interaction of biotic factors of the environment (Boring et al., 1981; Aksamit and Irving, 1984).

Realizing the overarching values of forests and considering their depletion at unprecedented rate, conservation of forests has emerged as the prime objective. As such, it is globally accepted that the depletion of forests has many ecological, social and economic consequences; one among these is loss of biodiversity (Jha et al., 2000). Forests form the renewable natural resource on earth and occupy very unique position among the various natural resources by supporting life on earth in several ways and providing services that cannot be substituted by any other means.

Since its inception (1988), the diverse ecosystems and their components in Nanda Devi Biosphere Reserve (NDBR) have remained attraction of researches. The representative ecosystems and their components in the reserve have shown evidences of change with time and space. In particular, the variations and changes in plant communities have been reported to be highly dependent on geographical, environmental and anthropogenic factors. Besides, differences in soil parameters, fire intensity, over harvesting and other kinds of disturbances contribute to the variation in vegetation from one stand to another or even within a community. Therefore, the reserve management, most often, looks for authentic and precise information on structure and composition of vegetation so as to address diverse issues of conservation and management at different levels ranging from species and community to landscape level. The forests in NDBR not only form diverse representative ecosystems but also are the home for many rare and endangered species. While the core zone of reserve consists of 10% forests, the buffer zone has nearly 27% area under forests. These frosts help in maintaining rich floral (angiosperms- 699, gymnosperms- 11, pteredophytes- 137, bryophytes- 146, lichens- 77 and fungi- 128 spp.) and faunal (mammals- 29, birds- 243, insects- 229, molluscs- 14, amphibian- 8, annelids- 6, reptiles- 3 and pisces- 1) diversity in the reserve (Rawal and Rawat, 2012). In recognition of its uniqueness, NDBR has been included in World Network of Biosphere Reserves (WNBR) by UNESCO since 2004. Also, the Nanda Devi and the Valley of Flowers National Parks, forming core zone of the reserve, have been inscribed on the World Heritage List by UNESCO under Natural Criteria vii and x.

Though, studies on different aspects of biodiversity have been carried out in NDBR of Himalaya viz. natural resource utilization (Joshi, 2002; Joshi and Samant, 2004; Silori, 2001), ecosystem functions (Adhikari, 1992; Garkoti, 1992, 2008; Singh et al., 1994; Garkoti and Singh, 1995; Gairola, 2005), management and development (Rawal and Rawat, 2012), threat assessment (Kala et al., 1998; Kala, 2005; Joshi, 2002; Joshi and Samant, 2004), ethonobiological enumerations (Joshi et al., 2000; Kala, 2005; Rawat et al., 2013) and floristic analysis (Joshi, 2002; Gairola, 2005; Sekar and Rawat, 2011; Rawat, 2013) but a systematic approach on the population structure and seasonal regeneration pattern of forest communities in NDBR with respect to their long term existence, is still lacking. Under the provision of protected areas, the need for understanding the structure and regeneration pattern in forests have been already emphasized to mitigate the ongoing challenges like overexploitation, deforestation etc., that emerged along with the present changing climate and socio-economic scenario.

Therefore, an understanding of the processes that affect regeneration of forest species is of crucial importance to both ecologists and forest managers in protected areas. Keeping the above in mind, seasonal phytosociological investigations have been carried out in a part of Nanda Devi Biosphere Reserve of Uttarakhand.

MATERIALS AND METHODS

Study area and site selection

The Nanda Devi Biosphere Reserve (NDBR), which forms the extensive study area, was designated as Biosphere Reserve by Government of India on 18th January, 1988. The reserve has a unique combination of diverse ecosystems including traditional agro ecosystems, various types of temperate forests, alpine meadows, glaciers, etc. It represents the west Himalayan highland (2b) province of the biogeographic zone-Himalaya and lies between

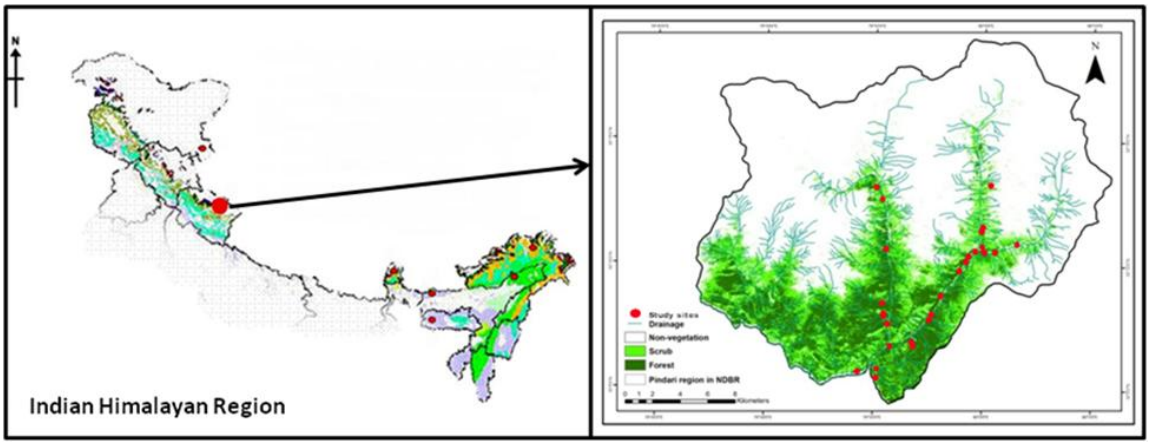

Figure 1. Location of study site of Pindari-Sunderdhunga-Kafni (right) in Nanda Devi Biosphere Reserve, western Himalaya (left).

30°06' and 31°04' north latitude and 79°13' and 80°17' east longitudes (Figure 1), and covers a total of 6,407.03 km^2 (Core zone 712.12 km^2; Buffer zone 5,148.57 km^2, Transition zone 546.34 km^2). One representative sites (Pindari-Sunderdhunga-Kafni: PSK in Kumaun region) in the buffer zone of NDBR formed the intensive study sites. Extensive surveys were conducted during 2008-2012 in these sites.

Sampling and data collection

Attempt was made to reach maximum possibly approachable stands. Standard phytosociological methods were followed to obtain quadrat data (Grieg-Smith, 1957; Misra, 1968; Kershaw, 1973; Muller-Dombois and Ellenberg, 1974; Dhar et al., 1997). In general, from each quadrat, circumference at breast height (CBH at 1.37 m from the ground) of all tree individual was recorded. Based on this information, individuals were considered as tree >31 cm; sapling 11-30 cm; seedling <11 cm CBH. Further, the individuals of tree species were grouped into six arbitrary CBH classes (A: <10; B: 11-30; C: 31-60; D: 61-120; E: >120 cm). The total number of individuals, belonging to each of the above classes, was calculated for each species in individual stand and stand information was pooled to represent community. Size class A and B represented seedlings and saplings, respectively. Other classes (C-E) represented tree classes. Relatively density of species in a particular size class was calculated as a percentage of total number of individuals in all size classes.

The quadrat information was pooled for calculating density, frequency, total basal area and their relative values (Misra, 1968; Muller-Dombois and Ellenberg, 1974). Following Whittaker (1975) and Pielou (1975), species richness was considered simply as the number of species per unit area. Species diversity index was computed using Shannon-Wiener information function (Shannon and Weiner, 1963). Statistical analysis (*t*-test and correlation coefficient (r) and coefficient determination (r^2)) and similarity indices were calculated using SPSS version 16 to determine the relationship between different phytosociological parameters.

Community structure and regeneration patterns

Seasonal investigation (Negi, 1995) on population structure and regeneration behavior of all tree species in PSK site was carried out during summer season: May-June, rainy season: mid July to August and winter season: November-December in the years 2009 and 2010. Eleven representative forest communities were identified for studying detailed population structure to predict the future compositional changes in parent communities.

Regeneration status of species was determined based on population size of seedlings and saplings (Khan et al., 1987; Shankar, 2001; Bhuyan et al., 2003): good regeneration, if seedlings > saplings > trees; fair regeneration, if seedlings > or ≤ saplings ≤ trees; fair regeneration, if the species survives only in sapling stage, but no seedlings (saplings may be <, > or = trees). If a species is present only in tree form, it is considered as not regenerating, while species having no trees but only seedlings is considered as 'new' species.

RESULTS

Site characteristics, floristic diversity and demographic patters

Eleven representative forest communities were distributed between 2025 to 3343 m asl. The community types, site representation and important species (IVI) are presented in Table 1. In general, 332 plant species were recorded from target site in NDBR. Of these, greater proportion (70.8%; 235 spp.) was of herbs. Shrubs constituted 16.3% (54 spp.), and trees 13.0% (43 spp.). Considering various taxonomic groups of the total 332 species in PSK site, 88.9% were angiosperms, 1.2% gymnosperms and 9.9% pteridophytes.

Proportional distribution of individuals in three broad tree strata (tree, sapling and seedling) and life forms (tree, shrubs, and herbs) in the study area and representative sites are depicted in Figure 2. In general, PSK site represented 43 tree species. Representation as compared to total tree species across different strata indicated maximum proportion of trees (93.2%) followed by saplings (81.4%) and seedlings (74.4%). The overall population structure for target sites and the entire reserve have been presented (Figure 3). As reflected, PSK site

Table 1. Site characteristics and dominant species across forest communities in PSK site.

Community types (PSK site)	Altitude (masl)	Slope (°)	No. of stands	Important species (IVI value)
Alnus nepalensis (Utis)	2025	40-45	2	*Alnus nepalensis* (128.2) *Ulmus wallichiana* (32.9)
Mixed Oak- Deciduous	2217	40-55	3	*Quercus floribunda* (79.8) *Aesculus indica* (34.6)
Hippophae salicifolia (Chuck)	2452	5-15	3	*Hippophae salicifolia* (232.0) *Alnus nepalensis* (32.6)
Quercus floribunda (Tilonj Oak)	2504	35-50	4	*Quercus floribunda* (127.1) *Rhododendron arboreum* (42.7)
Quercus semecarpifolia (Kharsu Oak)	2669	35-50	4	*Quercus semecarpifolia* (112.7) *Rhododendron arboreum* (37.9) *Quercus floribunda* (35.3)
Mixed-deciduous	2773	40-55	3	*Acer cappadocicum* (49.4) *Ulmus wallichiana* (28.1) *Rhododendron arboreum* (26.9)
Mixed Silver fir-Oak	2855	30-45	2	*Abies pindrow* (68.5) *Quercus semecarpifolia* (33.8) *Aesculus indica* (30.4)
Mixed Silver fir-Rhododendron-Maple	2860	40-50	3	*Rhododendron barbatum* (74.0) *Abies pindrow* (38.9) *Ilex dipyrena* (27.5)
Abies pindrow (Silver fir)	2970	60-65	2	*Abies pindrow* (99.9) *Rhododendron barbatum* (47.6) *Betula utilis* (44.0)
Mixed Birch-Silver fir	3238	50-60	2	*Betula utilis* (126.4) *Abies pindrow* (67.9) *Taxus wallichiana* (32.6)
Betula utilis (Birch)	3343	50-65	2	*Betula utilis* (183.4) *Euonymous fimbriatus* (29.1) *Rhododendron campanulatum* (20.8)

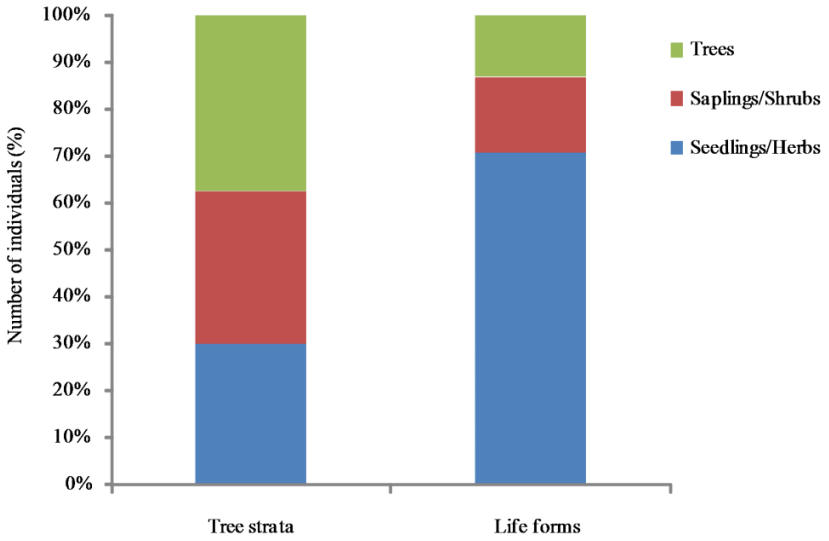

Figure 2. Proportional distribution of species richness across different tree strata and life forms in PSK site in NDBR.

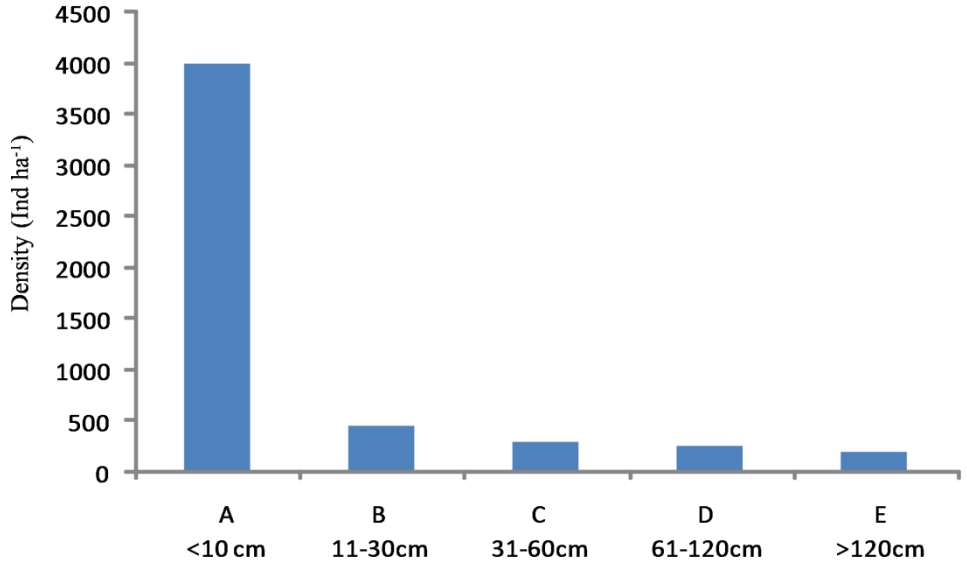

Figure 3. Mean density-diameter distribution of trees in PSK site.

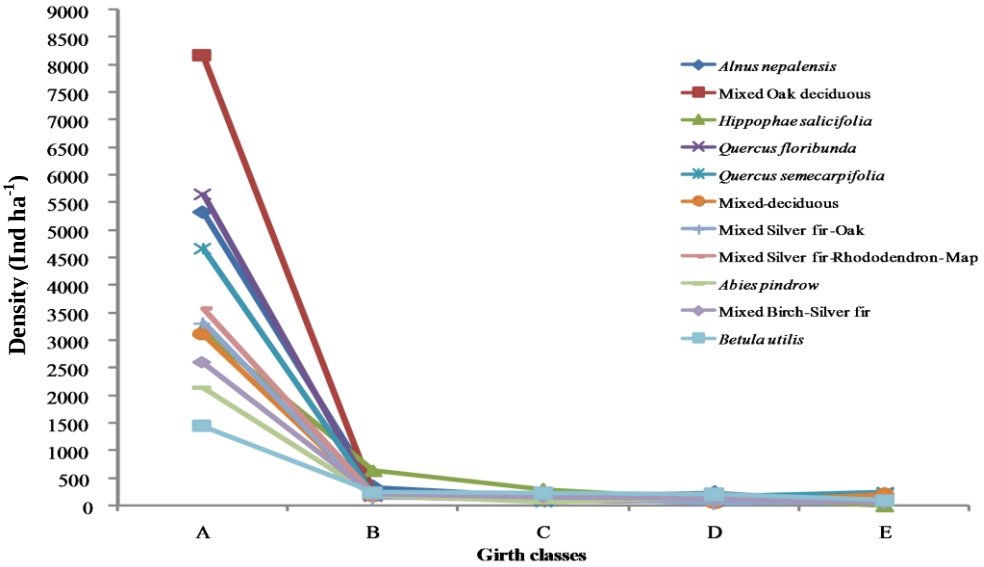

Figure 4. Density-diameter distribution of trees in different forest communities in PSK site.

has considerably larger number of individuals in seedling stage and more gradual decline of individuals towards higher tree size classes. The profile of demography for different forests in PSK site exhibited more or less similar patterns (Figure 4).

Community diversity and distribution pattern

Detailed quantitative ecological parameters in different forest communities are presented in Table 2. Considering the forest composition, tree species richness was highest in *Quercus semecarpifolia* and Mixed Silver fir-Oak communities (23 spp. each) and minimum in *Hippophae salisifolia* community (4 spp.). Sapling species richness peaked in *Q. floribunda* (18 spp.) followed by Mixed deciduous and Mixed Silver fir-Oak communities (15 spp. each). Lowest richness was recorded in *H. salicifolia* community (3 spp.). Species richness at seedling stage was maximum in *Quercus floribunda* (19 spp.) followed by *Quercus semecarpifolia* and MIXED deciduous community (16 spp. each) and lowest in *H. salicifolia* and *Betula utilis* communities (5 spp. each). The details of species richness in different tree layers and across

Table 2. Quantitative ecological parameters in different forest communities in PSK site.

Community types (PSK site)	Species richness			Species density (Ind ha^{-1})			Species diversity		
	Seedling	Sapling	Tree	Seedling	Sapling	Tree	Seedling	Sapling	Tree
Alnus nepalensis	9	9	14	5325± 878	345 ± 53	460± 49	2.20 ± 0.3	2.38 ± 0.3	2.38 ± 0.2
Mixed Oak deciduous	13	12	21	8170± 3012	233 ± 33	480 ± 50	2.28 ± 0.2	2.66 ± 0.1	3.37 ± 0.1
Hippophae salicifolia	5	3	4	3167 ± 609	633 ± 179	423 ± 174	0.70 ± 0.2	0.64 ± 0.3	0.73 ± 0.1
Quercus floribunda	19	18	20	5650 ± 1613	170 ± 27	378 ± 40	2.46 ± 0.4	2.48 ± 0.1	2.84 ± 0.2
Quercus semecarpifolia	16	14	23	4663 ± 819	200 ± 37	473 ± 42	2.27 ± 0.3	2.10 ± 0.3	2.73 ± 0.4
Mixed-deciduous	16	15	21	3117 ± 348	197 ± 41	407 ± 73	2.34 ± 0.3	2.47 ± 0.2	3.31 ± 0.1
Mixed Silver fir-Oak	9	13	21	3325 ± 469	145 ± 12	260 ± 16	2.27 ± 0.1	2.39 ± 0.1	3.20 ± 0.1
Mixed Silver fir-Rhododendron-Maple	14	15	23	3583 ± 866	200 ± 26	367 ± 39	2.51 ± 0.1	2.67 ± 0.3	3.22 ± 0.3
Abies pindrow	9	10	17	2150 ± 653	190 ± 8	320 ± 49	2.08 ± 0.4	2.55 ± 0.1	2.71 ± 0.2
Mixed Birch-Silver fir	9	11	10	2600 ± 694	210 ± 0	355 ± 102	2.49 ± 0.3	2.64 ± 0.1	2.02 ± 0.1
Betula utilis	5	6	8	1450 ± 531	235 ± 20	535 ± 116	1.50 ± 0.1	1.81 ± 0.3	1.55 ± 0.2

communities are presented.

The tree density ranged from 260 (Mixed Silver fir-Oak community) to 535 ind ha^{-1} in *B. utilis* community. In the case of saplings, maximum density was recorded in *H. salicifolia* community (633 ind ha^{-1}) and minimum in Mixed Silever fir-Oak community (145 ind ha^{-1}). Seedling density, however, peaked in Mixed Oak deciduous community (8170 ind ha^{-1}) followed by *Q. floribunda* (5650 ind ha^{-1}) and *Alnus nepalensis* community (5325 ind ha^{-1}). The minimum seedling density was recorded in *B. utilis* community (1450 ind ha^{-1}).

While considering the diversity index, highest value for tree layer was in the case of Mixed Oak deciduous community (3.37) followed by Mixed deciduous (3.31) and Mixed Silver fir-Rhododendron-Maple community (3.22). Whereas, Mixed Silver fir-Rhododendron-Maple community (2.67) followed by Mixed Oak deciduous (2.66) and Mixed Birch-Silver fir community (2.64) showed highest values in sapling layer. Mixed Silver fir-Rhododendron-Maple community (2.51) also peaked for seedling diversity followed by Mixed Birch-Silver fir (2.49) and *Q. floribunda* community (2.46). *H. salicifolia* community invariably had lowest diversity values across three tree strata (tree - 0.73, sapling - 0.64, seedling - 0.70).

Regeneration status and seasonal behavior

In the target site, out of the 43 tree species, 16.3% showed good, 46.5% fair, 25.6% no, 7.0% poor regeneration and remaining 4.7% were represented only by seedlings and saplings (Table 3). ANOVA based analysis revealed uneven variation in density values across the seasons (Figure 5). Starting from summer (2009), a significant (p>0.05) increase in the number of seedlings was observed with onset of rainy season (2009).

Afterward, the seedling density gradually decreased in winter (2009) and summer (2010) and increased significantly in the next rainy season (2010). In the year 2009, the average seedling density was measured about 2,867 ind ha^{-1} in summer that reached 3,491 ind ha^{-1} in the rainy season. In winter 2009, gradual decrease in seedling density (3,182 ind ha^{-1}) was recorded. Similar trends were observed in 2010. A linear regression line showed gradual but non-significant (p>0.05) increase in seedling density across the years.

The growth and establishment of seedling is irrespective of the altitude (Figure 6). *A. nepalensis* and mixed oak deciduous in lower altitudinal zone, Abies-Rhododendron-Maple in mid altitudinal zone and *B. utilis* in high altitude zone showed remarkable regeneration and seedling establishment.

DISCUSSION

Compositional diversity

Considering the floristic richness of representative

Table 3. Density and regeneration status in different forest communities in PSK site.

Species	No. of individuals ha^{-1}			
	Seedling	Sapling	Tree	Status
Abies pindrow	2937.5	109.2	297.5	Fair
A. spectabilis	-	5.0	10.0	No
Acer acuminatum	362.5	35.0	49.2	Fair
A. caesium	545.8	33.3	61.7	Fair
A. cappadocicum	1916.7	120.8	165.8	Fair
Aesculus indica	-	7.5	101.7	No
Alnus nepalensis	1970.8	220.0	274.2	Fair
Betula alnoides	1029.2	23.3	84.2	Fair
Betula utilis	1125.0	185.0	570.0	Fair
Buxus wallichiana	58.3	10.0	-	New
Carpinus viminea	154.2	14.2	66.7	Fair
Celtis australis	-	-	10.0	No
Cornus macrophylla	-	3.3	45.0	No
Corylus jacquemontii	50.0	10.0	33.3	Poor
Elaeagnus parvifolia	-	-	10.0	No
Euonymus fimbriatus	675.0	83.3	115.0	Fair
Eurya acuminate	-	5.0	-	No
Fraxinus micrantha	-	-	35.8	No
Hippophae salicifolia	3654.8	546.7	346.7	Good
Ilex dipyrena	1941.7	118.3	102.5	Good
Juglans regia	16.7	-	42.5	Poor
Lyonia ovalifolia	1170.8	51.7	124.2	Fair
Mahonia borealis	33.3	-	5.0	Poor
Meliosma dilleniaefolia	287.5	34.2	31.7	Good
Neolitsea pallens	3929.2	126.7	123.3	Good
Prunus cornuta	-	-	35.0	No
Pyrus lanata	387.5	12.5	37.5	Fair
Pyrus pahsia	12.5	6.7	-	New
P. vestita	83.3	5.0	61.7	Poor
Quercus floribunda	6037.5	64.2	287.5	Fair
Q. incana	-	-	11.7	No
Q. semecarpifolia	3037.5	19.2	194.2	Fair
Rhododendron arboretum	5820.8	272.5	310.0	Fair
R. barbatum	4887.5	225.8	267.5	Fair
R. campanulatum	650.0	53.3	65.0	Fair
Rhus punjabensis	-	-	2.5	No
Salix daphnoides	628.6	70.0	96.7	Fair
Symplocos chinensis	258.3	49.2	70.8	Fair
S. ramosissima	-	3.3	33.3	No
Syringa emodi	183.3	32.5	8.3	Good
Taxus wallichiana	912.5	116.7	114.2	Good
Ulmus wallichiana	633.3	35.8	152.5	Fair
Viburnum nervosum	487.5	64.2	2.5	Good

site, the PSK site with over 332 plant species emerged as more species rich and representative as compared to other sites in NDBR (Rawat, 2013). This can be attributed to the existence of more diverse and broadleaf forest communities (11 forest communities). In general, PSK site has 72% coverage of broadleaf dominated forests and nearly 28% conifer dominated ones, which is much higher than any other site in NDBR (Joshi, 2002; Joshi

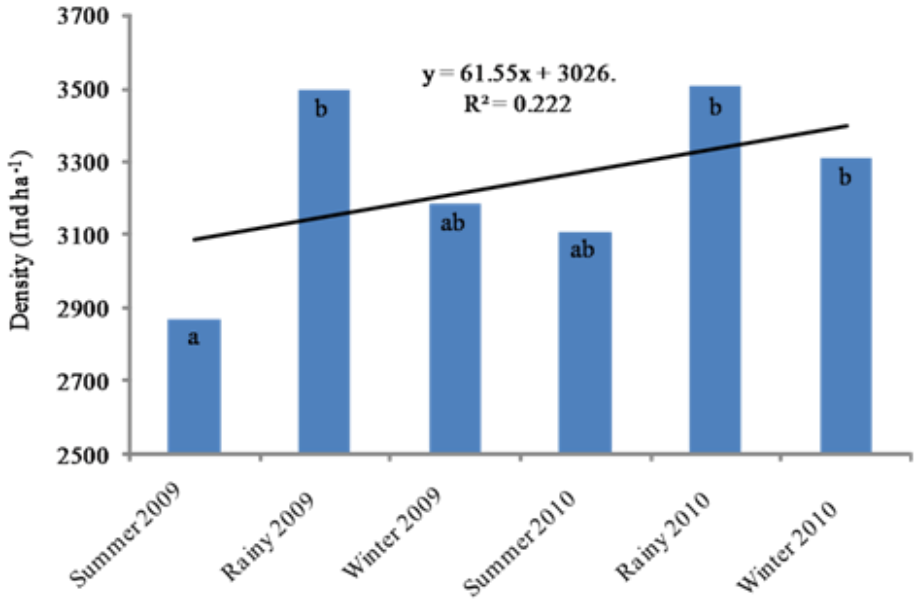

Figure 5. Average seasonal recruitment pattern in the entire forest communities. ANOVA was applied against the average density; same letters denote non-significant difference.

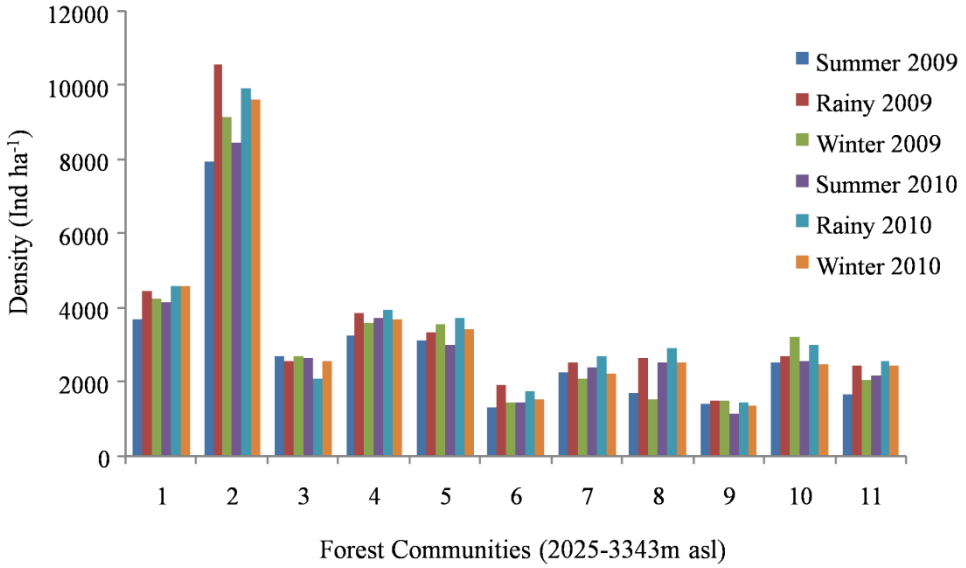

Figure 6. Seasonal recruitment patterns in different forest communities in PSK site in NDBR. [1. *Alnus nepalensis*; 2. Mixed Oak-deciduous; 3. *Hippophae salicifolia*; 4. *Quercus floribunda*; 5. *Quercus semecarpifolia*; 6. Mixed-deciduous; 7. Mixed Silver fir-Oak; 8. Mixed Silver fir-Rhododendron-Maple; 9. *Abies pindrow*; 10. Mixed Birch-Silver fir; 11. *Betula utilis*].

and Samant, 2004; Rawat, 2013). The explanations for this lies in the fact that PSK site supports more mesic (moist) conditions. In general, coniferous communities are broadly reported to be species poor as compared to broadleaf communities (Singh and Singh, 1992).

The mean tree density (260-535 ind ha^{-1}) was comparable to the values (320-1670 ind ha^{-1}) reported in earlier studies pertaining to low and high altitude forests of west Himalaya (Bankoti, 1990; Joshi and Samant, 2004; Gairola et al., 2008; Garkoti, 2008). The density values closely corresponded with the values (270-610 ind ha^{-1}) recorded from the same region two decade ago (Bankoti, 1990), however, these values fall in lower range of values reported for the region (Zobel and Singh,

1997).

The mean values range for seedling density (1450-8170 ind ha^{-1}) was considerably higher as compared to earlier reports for high altitude forest in similar areas (Bankoti, 1990; Joshi, 2002; Joshi and Samant, 2004). The range of mean sapling density (145-633 ind ha^{-1}) falls within the lower range (40-6667 ind ha^{-1}) reported earlier (Bankoti, 1990; Joshi, 2002; Joshi and Samant, 2004; Gairola et al., 2008).

Current state of tree species richness (4-23 species) was comparable to earlier reports (24-42 species) from high altitude forests of the region. Similarly, the diversity values (0.73-3.37) are within the reported range (0.45-3.29) from the region (Bankoti, 1990; Adhikari et al., 1991; Joshi, 2002; Gairola, 2005; Gairola et al., 2008). The trends of species richness and diversity indicate forests in mid to higher altitudes are more diverse than the lower altitude forests in the west Himalaya.

Current demographic profiles

The size class distribution gives a demographic profile of the region which may indicate future prospects of target communities (Gairola, 2005). In general, the forest communities showed greater accumulation of individual in seedlings and a significant decline towards sapling and tree size classes. This structure reveals that the conversion from seedling to sapling is not proportional. This can be explained on account of greater mortality of seedlings due to severe winters. Similar conclusions have been drawn by other workers elsewhere (Khumbongmayum et al., 2006).

Further, large scale extraction of biomass, particularly of selected species, has also been reported to bring in structural changes in plant communities in the region and elsewhere (Thadani and Ashton, 1995; Singh et al., 1997; Spurr and Barnes, 1980; Cairns and Moen, 2004; Shrestha et al., 2007). As such, disturbances have been observed to exert profound effect on forest development, since they alter vegetation and release growing space, making it available for other species to occupy (Oliver and Larson, 1990; Mishra et al., 2003; Mishra et al., 2004; Gairola et al., 2008). The lower density of the higher girth classes of trees in the region, as compared to intermediate or lower girth classes, can be attributed to the relatively high mortality of large canopy trees (Goff et al., 1975; Lorimer et al., 2001).

Seasonal regeneration pattern

While considering the demographic profile, greater accumulation of seedlings can be attributed to occasional mast seedling and recruitment for some dominant species (*Q. floribunda, Q. semecarpifolia*). However, the long-term persistence of such recruits was later confirmed by two years seasonal investigation on seedling survival patterns. Therefore, if this trend continues, the forest communities are likely to have increased dominance of such species.

Further, the seasonal recruitment patterns suggest that the regenerating species are now established and if this trend continues, the forest communities will sustain long in future. The overall population structure of tree species reveals that seedlings populations dominate tree populations and the fluctuation in population density in various seasons is related to the prevailing environmental factors. Germination of freshly dispersed seeds is high for most of the species during the rainy season, which is the wettest season. Lieberman and Li (1992) and Swaine et al. (1990) have observed similar patterns in tropical dry forest at Pinkwae, Ghana. Adverse effects of soil moisture stress and unfavorable temperature on survival of plant species may be responsible for reduction of seedling population during winter season (Perira and Kozlowski, 1977; Schulte and Marshall, 1983; Khumbongmayum et al., 2006). The gradual decrease in recruitments in summer season can be attributed to the anthropogenic pressure in form of lopping and grazing. Evolutionary history of grazing and environmental moisture or primary productivity interacts in determining species adaptations for tolerance or avoidance of herbivores and in community responses to grazing (Milchunas and Lauenroth, 1993). The average of fluctuations in recruitment density across altitude and seasons revealed established regeneration.

Expected changes in forests vis- a-vis representativeness

Broadly, the demographic profiles exhibited progressive structures suggesting long term persistence of the communities/species in these sites. However, diverse trends of density and richness of recruits helps us to depict the status of species in different forest communities (Khan et al., 1987; Shankar, 2001; Bhuyan et al., 2003). In this respect, following patterns across recruitment layers are noticeable for different communities:

Sapling layer

1. Communities having high representation of dominant species: *Alnus nepaensis, H. salicifolia,* Mixed deciduous, Mixed Silver fir-Rhododendron-Maple, *B. utilis* communities.
2. Communities having high representation of co-dominant species: Mixed Birch-Silver fir
3. Communities having poor representation of dominant species but highest of co-dominant species: *Q. floribunda, Q. semecarpifolia* communities.

4. Communities having poor representation of dominant and co-dominant species and high representation of other species: Mixed Oak deciduous**,** Mixed Silver fir-Oak communities.

Seedling layer

1. Communities having high seedling representation of dominant species: Mixed Oak deciduous, *H. salicifolia, Q. floribunda, Q. semecarpifolia,* Mixed Oak deciduous, Mixed Silver fir-Oak, Mixed Silver fir-Rhododendron-Maple, *Abies pindrow, B. utilis* communities.
2. Communities having sufficient representation of both dominant and co-dominant species, and accompanied by high representation of other species as well: Mixed Birch-Silver fir community.

Therefore, based on above trends of seedlings and saplings, various combinations and trends of communities can be drawn. For example, (i) the communities with greater representation of dominant species in both seedling and sapling stage would suggest further strengthening of dominant species; (ii) the communities with greater representation of both dominant and co-dominant in sapling and seedling layers would indicate the composition remains unchanged in future; (iii) the communities having greater proportion of seedling and saplings of co-dominant would indicate possible dominance of such species in future; (iv) the communities having greater representation of seedlings and saplings of the species other than the dominants and the co-dominants would indicate likely future changes in composition of target communities.

The demographic profiles of some of the dominant and some relatively less prominent tree species definitely require attention. For example, in the case of two dominant species, *Q. floribunda* and *Q. semecarpifolia*, in spite of their greater seedling numbers both were less prominently represented in sapling layer. Certain relatively less prominent species like *Celtis australis, Elaeagnus parvifolia, Fraxinus micrantha, Prunus cornuta, Quercus incana* and *Rhus punjabensis* were, however, represented only in tree layer suggesting that these species are not properly regenerating through near past and in the present. Therefore, long-term persistence of such species is in question. Besides, *Abies spectabilis, Aesculus indica, Symplocos ramosissima* with representation only in tree and sapling class and *Cornus macrophylla, Juglans regia, Mahonia borealis, Viburnum nervosum* in tree and seedling class only would require attention. On the contrary, species like *Buxus wallichiana, Pyrus pahsia, Eurya acuminata* having individuals in sapling and seedling class only indicated their recent introduction in respective communities.

Differences in regeneration behavior of various species are indicative of future structure and dynamics of the communities under natural circumstances. Present study reveals good regeneration and exemplifies regeneration of tree species which is largely dependent on the prevailing environmental factors and anthropogenic threat, and if the existing ecological factors are not jeopardized, the future maintenance of the tree species in PSK site in NDBR will be sustained. However, presence of 'new' and 'not regenerating' species must be taken into consideration at the time of development of policies and plan for proper conservation and management of respective forest communities.

Conclusion

1. The data sets available, and generated through this study, provide enough bases for establishing PSK site in NDBR as potential sites for long-term ecological monitoring under various change scenarios.
2. Comparatively, the PSK site of the reserve supported greater diversity of plant communities and species as compared to other sites in the reserve.
3. Communities in target site broadly exhibited progressive demographic profiles which suggested long-term persistence of communities. However, unusually greater accumulation of seedling in PSK site with indications of successful establishment is indicative of possible changes in composition of communities in this site. Also, various community specific patterns of demography were revealed.
4. Assessment and analysis of changes in structure and composition of different forest types provides baseline data for developing priorities for conservation of other representative landscapes in the reserve as well as in the Himalaya.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Chemical characterization and antimicrobial activity of essential oils and Croton's varieties modulator in the Brazilian's Northeast semiarid

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This study aimed to analyze the chemical composition and evaluate the antibacterial activity of essential oils and modulator of *Croton heliotropiifolius* Kunth and *Croton blanchetianus* Baill. The oils were obtained with distilled water and their components identified with GC/MS. Eucalyptol (16.9%), β-caryophyllene (15.9%) and germacrene-D (14.5%) for *C. heliotropiifolius* and cedrol (28.4%), eucalyptol (17, 4%) and α-pinene (10.5%) for *C. blanchetianus* were observed. The antimicrobial activity and minimum inhibitory concentration (MIC) was determined by the diffusion method and microdilution with standard bacteria Gram positive and negative. Preliminary results of the antibacterial activity showed that both oils were more effective against Gram-positive strain of *Bacillus cereus*. When verifying the minimum inhibitory concentration, it was observed that the essential oil of *C. heliotropiifolius* showed inhibitory activity only for the lineage multiresistance *Staphylococcus aureus* (MR 358), 512 µg/mL with MIC. For the essential oil of *C. blanchetianus*, the result was significant for *S. aureus* with an MIC of 64 mg/mL. The oil of *C. blanchetianus* potentiated the antibiotic amikacin, kanamycin and gentamycin against *B. cereus* strain, showing a synergistic effect.

Key words: Phytochemistry, antimicrobial, bacterial resistance, secondary metabolites.

INTRODUCTION

A significant number of bacterial populations have acquired resistance to antimicrobial. Antibiotics are important therapeutic agents commonly used for the control of bacterial infectious diseases. However, resistance to antibiotics has become a global public health problem. Bacterial infections caused by resistant strains are problems in

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numerous hospitals around the world, especially in patients compromised by age, illness and treatments with immunity-suppressant drugs (Lima et al., 2013). The knowledge on the therapeutic properties of medicinal plants obtained from the folk medicine, has been accumulated over centuries and the empirical knowledge often symbolizes the only therapy of various communities and ethnic groups. In Brazil, medicinal plants represent a rich source of which antimicrobial agents may be obtained. Plants are used medicinally in different infections and are a source of many potent and powerful drugs (Kubmarawa et al., 2007; Almeida et al., 2013).

Croton blanchetianus and *Croton heliotropiifolius,* popularly known as “velame-do-campo”, are shrub widely distributed in Brazilian north east occurring in vegetations (Ceará, paraíba and Pernambuco) (Gomes, 2006).

The interest on plants for investigations of new antimicrobials is due to the wide variety of chemical substances present in different parts of the plants with pharmacological action, as coumarin, flavonoids, terpenoids, alkaloids and tannins. In this sense, the most recent research involves screening of plant extracts and essential oils, to thus meet secondary metabolites with relevant biological activity. Presented for the first time in this work is the chemical composition of essential oil of *C. blanchetianus and C. heliotropiifolius* varieties obtained from the Caatinga`s biome, and antimicrobial modulatory activities against bacterial strains.

MATERIALS AND METHODS

The leaves of *C. blanchetianus* and *C. heliotropiifolius* were collected in October and November 2009 at 8:00 p.m. at the site “São José do Bofim” municipality of Patos-PB, between geographic coordinates of latitude 070 08'20 9 "and longitude 0370 18 '062". Then a representative sample of each species was identified by Professor Maria de Fátima Souza and deposited at the Caatinga`s Herbarium in the Federal University of Campina Grande (UFCG), under registration number # 496 and # 497, respectively.

The essential oils from fresh leaves of *C. blanchetianus* (820.0 g) and *C. heliotropiifolius* (652.95 g) were obtained by hydrodistillation using handset type Clevenger, for a period of two hours. Then the oil was dried with anhydrous sodium sulfate to remove excess water and kept in the refrigerator within 30 days until analysis. The identification of the essential oils chemical components of *C. blanchetianus* leaves and *C. heliotropiifolius* were obtained by gas chromatography-mass spectrometry (CG/EM), in a Hewlett-Packard model 5971 spectrometer operating at 70 eV ionization energy. Fused silica capillary column DB-5 (30m x 0.25 mm d.i., 0.25 µm film thickness) and helium carrier gas stream 1 mL/min com split were used. The injector and detector temperatures were programmed from 250 to 200°C, respectively. The column temperature was programmed 35 and 180°C at 4°C/min and, then the 180°C at 280°C at 10°C/min. Mass spectra were obtained at 30 and 450 m/z. Individual components were identified by matching their mass spectra, 70 eV, with the database, using the library built and spectrometer with two other computers using retention indices as a pre-selection as well as by visual comparison of the fragmentation pattern with those reported in the literature.

Five standard bacterial strains used were ceded by the Oswaldo Cruz Foundation for the preliminary assessment of the antibacterial activity of three Gram-negative: *Pseudomonas aeruginosa* (15442), *Klebsiela pneumoniae* (10031) and *Escherichia coli* (25922), two Gram-positive *Staphylococcus aureus* (ATTCC 12692), *Bacillus cereus* (ATTCC 33018) and a multiresistant strain isolated from clinical material *S. aureus* (MR 358), which have been revived amid middle Brain Heart Infusion (BHI) and incubated for 24 h at 37°C.

The antibacterial activity was determined by agar diffusion method by gel cavity. The bacteria were inoculated, using a swab sterile in Petri dishes previously prepared with Agar Muller-Hilton made cavities which (6 mm in diameter) is filled with 20 µL prepared with the essential oils of the plants solutions (diluted in DMSO) in the following concentrations: 10; 5; 2.5; 1.25; 0.6 and 0.3%. Then the plates were incubated at 37°C on the bacterial incubator for 24 h. Assays were performed in duplicate, together with the positive control antibiotic amikacin (30 µg) and chloramphenicol (30 µg) and as a negative control DMSO and distilled water. It was considered as the final result of each sample of the average of the measurements of the halos and susceptible to inhibition zone at or above 10 mm diameter corresponding to the diameter of the cavity in the culture medium.

The tested essential oils showed antimicrobial activity in the preliminary assessment submitted for the determination of minimum inhibitory concentration (MIC) by broth microdilution technique, based on the document CLSI/NCCLS M7-A6 to bacterium (NCCLS 2003).

The same pattern of the previous test strains, which were used, were inoculated in middle brain hear infusion broth (BHI) broth at 3.8% and incubated for 24 h at 35 ± 2°C. After this preculture, the standardization of the inoculum was done, which consisted of the preparation of a bacterial suspension in BHI at 3.8%, turbidity corresponding to 0.5 Scale McFarland (10^8 UFC/mL). Then, this suspension was diluted to 10^6 UFC/mL in broth BHI at 10%, and volumes 100 µL were then homogenized in 96 well microdilution plate supplemented with different concentrations of oil, resulting in a final inoculum of 5×10^5 UFC/mL.

Solutions of essential oils were prepared using 10 mg of samples solubilized in 1 mL of DMSO (SIGMA, Brazil) obtaining an initial concentration of 10 mg/mL. From this concentration, dilutions were made in distilled water to obtain a stock solution of 1024 mg /mL. Final concentrations of the oils in the culture medium were 512, 256, 128, 64, 32, 16 and 8 µg/mL.

The tests were performed in duplicate and the plates were incubated at 35 ± 2°C for 24 h. A developer was added to each well, 25 µL of resazurin (sodium resazurin SIGMA, Brazil) prepared in sterile distilled water at a concentration of 0.01% (w/v) for a period of 30 min at room temperature. The negative control was gotten with 100 µL BHI broth plus the standardized bacterial inoculum. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that is able to completely inhibit microbial growth in the microdilution wells as detected by the naked eye. The reading of the results for MIC determination was considered as positive for the wells that were blue and those who had negative staining, red coloring.

For the evaluation of the essential oil as a modulator of antibiotic activity, the MIC conventional aminoglycoside antibiotics (neomycin, kanamycin, amikacin and gentamicin), were determined in the absence and presence of the essential oil by the microdilution method, modified from the paper CLSI/NCCLS M7-A6 to bacterial (NCCLS 2003). Subinibitory concentrations were used (MIC 1/8) in BHI at 10%. Two strains of bacteria transferred patterns were used in the test by Oswaldo Cruz Foundation, with two Gram-positive *B. cereus* (ATTCC 33018) and *S. aureus* (ATTCC 12692). The standard strains were selected because of the essential oil of *C. blanchetianus* providing the lowest MICs in antibacterial assays.

Solutions of the antibiotics were prepared with addition of distilled water to obtain a concentration corresponding to 1024 µg/mL. A

Table 1. Chemical constituents identified in the essential oils from the leaves of *C. heliotropiifolius* and *C. blanchetianus*.

Constituent	*C. heliotropiifolius*		*C. blanchetianus*	
	RI	%	RI	%
α-Pineno	939	**3.5**	939	10.5
ß-Pineno	-	-	980	3.0
Sabineno	976	2.4	-	-
ß-Mirceno	991	5.5	991	1.5
p-Cimeno	1026	5.4	1026	4.2
Eucaliptol	1033	16.9	1033	17.4
γ-Terpineno	1062	4.5	-	-
Linalool	1098	1.1	1098	1.5
Acetato de bornila	1285	3.5	-	1.3
Acetato de terpinila	1346	1.7	-	-
α-Copaeno	1346	1.7	-	-
ß-Cariofileno	1418	15.9	1418	3.8
α-Humuleno	1452	2.1	1452	1.3
Germacreno D	1480	14.5	-	-
Biciclogermacreno	1494	10.4	-	-
δ-Cadineno	1524	1.1	-	-
Espatulenol	1576	3.7	1576	2.8
caryophyllene oxide	1581	1.7	1581	1.2
Viridiflorol	1590	1.2	-	-
Cedrol	-	-	1589	28.4
Alloaromadendreno	-	-	1458	1.2
p-Cimen-8-ol	-	-	1189	1.3
Criptona	-	-	1186	1.3
Monoterpenos	-	13.2	-	39.2
Sesquiterpenos	-	80.7	-	10.3
Total identified		98.8%		79.4%

*Relative retention Indices (literature values).

volume of 100 mL of each solution of antibiotics was diluted seriously with wells containing BHI broth and 10% bacterial suspension diluted (1:10). Finals concentrations of the antibiotics in the culture medium was 512, 256, 128, 64, 32, 16, 8, 4 and 2 µg/mL. After incubation of the plates at 37°C for 24 h, the essential oils' action on the effect of antibiotics was demonstrated by the use of resazurin as specified above.

RESULTS AND DISCUSSION

The essential oils obtained with the leaves hydrodistillation showed yields of 0.075% and 0.72% for *C. heliotropiifolius* and *C. blanchetianus* relative to the weight of the fresh material used. By analyzing CG-EM it was possible to identify and quantify the constituents 23 (Table 1). The essential oil of *C. heliotropiifolius* constituents 18 account for 98.8%, with 13.2% monoterpenes and 80.7% identified sesquiterpenes. Among the compounds identified were eucalyptol (16.9%) as major compound followed by β-cariofilene (15.9%) and germacreno-D (14.5%). 15 components were identified, *C. blanchetianus* (79.4%), being 39.2% monoterpenes and 10.3% sesquiterpenes constituents. The major compounds were cedrol (28.4%), eucaliptol (17.4%) and *α*-pineno (10.5%). The chemical profile of the oils was similar, but showed a different proportion species showing a high amount of oil of sesquiterpenes for *C. heliotropiifolius*, whereas for *C. blanchetianus* there is a predominance of monoterpenes. However, this chemical composition is consistent with literature data for Croton species whose essential oils are characterized by the predominance of monoterpenes and sesquiterpenes as major components. Silva (2008) with the essential oil extracted from the leaves of *C. heliotropiifolius* identified the presence of α-pinene, sabinene, linalool, bornila of acetate, beta-caryophyllene, germacrene D, δ-cadinene, α-humulene, bicyclogermacrene, spathulenol and eucalyptol as major compounds , corroborating the data of this study. According to Silva et al. (2010), the chemical composition of the essential oil of *C. sonderianus* presents the following constituents:

Table 2. Mean values of the halo of inhibition of bacterial growth in mm of the essential oil *C. blanchetianus*.

Mean of inhibition zones (mm diameter) (Mean + standard deviation)									
		Oil concentration (%)						Control	
Plants	**Bacterial**	**10**	**5**	**2.5**	**1.25**	**0.6**	**0.3**	**CLO**	**AMI**
C. h*	*P. aeruginosa*							23.5±2.1	23.5±0.7
	S. aureus	5.5±7.77						0.0±0.0	19.5±0.7
	S. aureus M.R							21.0±1.4	21.5±3.5
	E. coli							0.0±0.0	19.0±4.2
	K. pneumoniae	5±7.07						0.0±0.0	26.5±2.1
	B. cereus	11.5±2.12						17.5±0.7	22.0±4.2
C. b*	*P. aeruginosa*	-	4.5±6.36	-	-	-	-	23.5±2.1	23.5±0.7
	S. aureus	4±5.65	3.5±4.94	-	-	-	-	0.0±0.0	19.5±0.7
	S. aureus M.R	6.5±9.19	5±7.07	-	-	-	-	21.0±1.4	21.5±3.5
	E. coli	-	-	-	-	-	-	0.0±0.0	19.0±4.2
	K. pneumoniae	4.5±6.36	5±7.07	-	-	-	-	0.0±0.0	26.5±2.1
	B. cereus	13.5±4.94	8.5±0.7	5±7.07	3.5±4.94	-	-	17.5±0.7	22.0±4.2

*Concentration used: (CLO) chloramphenicol 30 µg; (AMI) amikacin 30 µg.

Table 3. Values in mg/mL minimum inhibitory concentration (MIC) of essential oils from leaves of *C. heliotropiifolius* and *C. blanchetianus.*

Bacterial	MIC (µg/mL)	
	EOCh*	**EOCb***
P. aeruginosa	≥ 1024	≥ 1024
S. aureus	≥ 1024	64
S. aureus M.R	512	≥ 1024
E. coli	≥ 1024	512
K. pneumoniae	≥ 1024	≥ 1024
B.cereus	≥ 1024	256

*EOCb: Essential oil of *C. blanchetianus* EOCh: Essential oil of *C. heliotropiifolius*.

spathulenol (18.32%), β-caryophyllene (14.58%) and caryophyllene oxide (8.54%), bicyclogermacrene (16.29%), β-phellandrene (15.42%) and β-caryophyllene (13.82%).

In this study, the essential oil extracted from the leaves of *C. heliotropiifolius* (Table 1) showed the presence of α-pinene, sabinene, linalool, bornila of acetate, beta-caryophyllene, germacrene D, δ-cadinene, α-humulene, bicyclogermacrene, spathulenol and eucalyptol as major compounds, corroborating the data of this study. The cedrol constituents, and alloaromadendreno criptona present in the essential oil of *C. blanchetianus* were only identified in this work, which may be related to the existence of chemotypes of this species found only in the Caatinga's biome.

As for the susceptibility testing (Table 2), for the preliminary determination of antibacterial activity, it was observed that both essential oils showed activity against the Gram-positive strain *B. cereus* (ATTCC 33018), with an average inhibition zone of 11.5 to 13.5 mm for *C. heliotropiifolius* and *C. blanchetianus* at a concentration of 10% as shown in Table 2. Gram-negative strains *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* multiresistant, were resistant to the essential oil of *C. heliotropiifolius* as there was no formation on a zone of inhibition.

For oil *C. blanchetianus*, the *E. coli* strain was resistant at all concentrations, whereas in the other ones, the inhibition zone was less than 10 mm showing a high resistance of these microorganisms to the action of the components present in the oil at the concentrations tested. However, the demonstrated effect of essential oils on the same line can be attributed to the proportion of all components present in the oils. Regarding the assessment of the minimum inhibitory concentration (MIC) (Table 3), it was found that the essential oil of

Table 4. Values of MIC (mg/mL) of aminoglycosides in the absence and presence of the essential oil from the leaves of *C. blanchetianus*.

Antibiotics	*S. aureus* (ATCC)		*B. cereus* (ATCC)	
	MIC	EOCb (64µg/mL)	MIC	EOCb (256 µg/mL)
Amicacine	8	8	8	4
Neomicine	32	32	8	8
Kanamicine	16	16	8	2
Gentamicin	2	2	4	2

*EOCb: Essential oil of *C. blanchetianus.*

C. heliotropiifolius showed inhibitory activity only for multidrug-resistant *S. aureus* strain (MR 358) with MIC 512 µg/mL. While the essential oil of *C. blanchetianus* showed inhibitory activity against *E. coli* strains (MIC 512 µg/mL), *B. cereus* (MIC 256 mg/mL) and *S. aureus* representing the most significant result with a lower MIC of 64 µg/mL. However, at the concentrations tested, the oils were not able to inhibit growth of the strains *K. pneumoniae* and *P. aeruginosa* MIC ≥ considering 1024 µg/mL.

Given the results, it is observed that the Gram-negative strains were resistant to the action of oils, mainly the species *C. heliotropiifolius*, thus, absence of this inhibitory activity of essential oils on the Gram-negative bacteria. Silva et al. (2007) perhaps showed that the existing structural differences between bacteria, and Gram-positive are considered more sensitive to exposure to antibacterial products while, in general, negative bacteria were more resistant to the action with this essential oil because they had a rich outer membrane lipopolysaccharide, responsible for the hydrophilic surface, hindering the penetration of hydrophobic substances such as the constituents of many essential oils. Gonçalves (2007) reported that the extract of *C. blanchetianus* showed resistance against the strains *E. coli*, *S. epidermidis, S. aureus and S. typhimurium*. For *C. heliotropiifolius,* germacrene-D identified as the major constituent, proved to be inactive against *Bacillus subtilis, B. cereus, S. aureus, P. aeruginosa* and *E. coli*, as assessed by the methods of diffusion Agar and microdilution broth (Deuschle et al., 2007).

Studies on *C. blanchetianus* and *C. heliotropiifolius* species with antibacterial activity are still lacking, probably due to the fact that these species are native to the Caatinga with seasonal variations and mainly produce oils with low income. However, antimicrobial activity with essential oils to other species of general Croton, as *Croton nepetaefolius*, *Croton tiglium* and *Croton lechleri* L. is observed (Froldi et al., 2009).

Due to the complex nature of the essential oils, it would be difficult to give attribute to the activity observed at any single component present in it. Therefore, to know the mode of action of the oils it would be necessary to examine separately each component of the essential oil and mixture to see if the chemical constituents have single or synergy inhibition.

The bacterial strains that showed the lowest inhibitory concentrations (MICs) were referred for modulating the activity of the essential oil against aminoglycosides.

In the result analysis (Table 4), it was observed that the essential oil of *C. blanchetianus* did not potentiate the activity of aminoglycoside antibiotics when tested against the *S. aureus* strain in 64 µg/mL concentration (MIC 1/8), the oil demonstrated a synergistic action on the activity of kanamycin, amikacin and gentamicin in the interaction with the strain *B. cereus*, with the most significant value of kanamycin with reduced MIC of 8 to 2µg/mL.

Oliveira et al. (2006) showed that the essential oil of some plants species in combination with the antibiotic gentamicin showed an antagonistic effect on *S. aureus* strain. Rodrigues et al. (2009) reported that the antibiotic activity gentamicin against *P. aeruginosa* was enhanced in the presence of the essential oil of *Croton zehtneri*, having a synergistic effect. However, the literature has not yet reported on the potentiating action of essential oils of the species studied with regards to the activity of aminoglycoside antibiotics.

Generally, the characteristics of the interference exerted by the essential oil on the antibiotic action varies according to the type of the antibiotic, the type of essential oil in combination tested, and the type of bacterial strain tested.

Conclusions

From the results obtained, it is concluded that the chemical constituents present in the essential oils of plants belonging to the class of monoterpenes and sesquiterpenes, have major compounds such as eucalyptus, β-caryophyllene and germacrene-D in the species *C. heliotropiifolius* and cedrol, eucalyptol and α-pinene in *C. blanchetianus*. Considering these results, we can concluded that the plants' essential oils when evaluated at a concentration of 10% have a higher antibacterial activity against the Gram-positive *B. cereus*

strain. Regarding the evaluation of the minimum inhibitory concentration, the essential oil of *C. heliotropiifoliu* is only active against the multidrug-resistant *S. aureus* strain with an MIC of 512 mg/mL. With the essential oil of *C. blanchetianus*, the antibacterial action with a MIC ranging 512-64 mg/mL proves to be more efficient for *S. aureus* (64 mg/mL). The essential oil of *C. blanchetinanus* acts synergistically with antibiotics amikacin, kanamycin and gentamicin against the strain *B. cereus*. Although essential oils do not present inhibitory activity against all pathogenic strains tested, these results are promising and indicate that they are a source of natural products that possess antibacterial activity thus providing an important contribution to enlarge the biological knowledge of the species.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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