

Original Research Article

Phytochemical Screening, Antioxidant and Antibacterial properties of *Taxillus cuneatus* (Roth.) Danser- A Hemi-parasitic Angiosperm

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ABSTRACT

Taxillus cuneatus (Roth.) Danser, an evergreen hemi-parasitic flowering plant belongs to the family Loranthaceae growing on *Spondias pinnata* (L.f.) Kurz was collected from Western Ghats of Karnataka and subjected for qualitative phytochemical analysis, antibacterial and antioxidant activity. In the present study, *T. cuneatus* was subjected for phytochemical analysis in different solvent extracts which revealed the presence of reducing compounds, alkaloids, flavonoids, saponins, tannins, sterols, terpenoids, cardiac glycosides, glycosides, carbohydrates and absence of anthraquinones, proteins and amino acids. The methanolic extract of the plant showed total phenolic content of about 33 µg of GAE in 100 µg of plant extract. Antioxidant and reducing power activities of methanol extract revealed dose dependent activity, which increased with increase in the concentration of the extract. Ethyl acetate and methanolic extracts exhibited moderate antibacterial activity against the tested bacterial pathogens. The present investigation has thrown a light on the effect of parasitic plant *T. cuneatus* on antioxidant and antibacterial activities, so it can be further subjected for purification of compounds which may act as an alternative for the present synthetic compounds which are used as pharmaceuticals.

Keywords

Taxillus cuneatus,
Secondary metabolites,
Total phenols,
DPPH,
Antibacterial

Introduction

Nature has been a source of medicinal agents for thousands of year because of their traditional medicinal practice (Mithraja et al., 2012). A remarkable number of modern drugs have been isolated from medicinal plants which have led to sudden increase in the number of herbal drug (Boopathi and Sivakumar, 2011). Medicinal plants with therapeutic properties are used for the treatment of

many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects (Sanaa et al., 2012). The medicinal properties of the plants are mainly due to the presence of secondary metabolites like alkaloids, cardiac glycosides, tannins, flavonoids, saponins, reducing compounds, minerals and vitamins (Vinoth et al., 2011). However,

the secondary metabolites are of great medicinal interest as they have significant biological activities and the actual active constituents of many crude drugs are still unknown.

Reactive oxygen species which create oxidative stress cause human diseases and disorders such as heart disease, inflammation, atherosclerosis, stroke, cancer, diabetes mellitus, malaria, HIV/AIDS, etc., (Rackova et al., 2007). Antioxidants derived from plants contain generally, the phenolics which have many biological activities such as anti-inflammatory, anti-cancer and anti-microbial (Gambhire et al., 2009; Mirzaei et al., 2013). Plants also have the capability to safeguard the body from oxidative damage by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes (Gyekyel et al., 2012). Due to the profitable efficiency of medicinal plants on biological activities, there is a need for isolation of newer biological compounds from plants which can serve as novel drugs.

Taxillus belonging to the family Loranthaceae has many medicinal values. Many species of *Taxillus* have medicinal values and are used in traditional medicine such as *T. theifer* (anti-hypertensive), *T. liquidambaricola* (antioxidant, analgesic and anti-inflammatory), *T. chinensis* (anti-obesity, anti-inflammation, antioxidant, immunomodulatory effects and anti-cancer), *T. sutchuenensis* (antioxidant, anti-inflammatory and antiproliferative) and *T. ramulus* (antioxidant, anti-inflammatory activity, neuroprotective, hypolipidemic effects, treatment of atherosclerosis and it prevent the renal toxic effects) (Chiu, 1996; Huang et al., 2008; Wang et al., 2008; Zhang et al., 2011; Deng et al., 2011; Lee et al., 2012;

Liu et al., 2012; Zhang et al., 2013). In the present investigation, *Taxillus cuneatus* (Roth.) Danser an parasitic angiosperm was subjected for its preliminary phytochemical analysis and other biological activities for the first time.

Materials and Methods

Collection of plant material

The fresh plant material (leaves) of *Taxillus cuneatus* growing on *Spondias pinnata* (Anacardiaceae) was collected from Western Ghats of Karnataka, India. The plant was identified with the help of Flora of Presidency of Madras (Gamble, 1935) and a voucher specimen is deposited in the Herbarium, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

Preparation of the extracts

The fresh leaves of *T. cuneatus* was washed under running tap water, shade dried and powdered using wearing blender. 50 gm of dried leaf powder was filled in the thimble and successfully extracted with petroleum ether; chloroform; ethyl acetate and methanol using Soxhlet extractor. All the extracts collected were concentrated using rotary flash evaporator and stored at 4° C in air tight vials and used for further studies (Harborne, 1973).

Phytochemical Screening

The collected plant extracts were subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents like reducing compounds, anthraquinones, alkaloids, flavonoids, saponins, tannins, cardiac glycosides, glycosides, sterols, terpenoids, carbohydrates, proteins and amino acids

using the method described by Harborne (1973) and Trease and Evans (1987).

Determination of Total Phenolics

Total phenolic in methanol extracts was determined by the method of Singleton et al (1999). 20 µl of extract (5 mg/ ml) was mixed with 0.75 ml of 20% sodium carbonate solution and 0.25 ml of Folin-Ciocalteu reagent. The reaction mixture was allowed to stand in light for 3 min and incubated for 2 h in dark. The absorbance was measured at 765 nm using UV-Visible Spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of known concentrations of Gallic acid standard (0-100 µg/ ml). The concentrations were expressed as µg of Gallic acid equivalents per ml.

Antioxidant activity by DPPH method

The free radical scavenging capacity of the methanolic extract of the plant was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Sultanova et al., 2001). The reaction mixture contained 5 µl of plant extract and 95 µl of DPPH (300 µM) in methanol. Different concentrations (100-1000 µg/ ml) of test sample were prepared, while the concentration of DPPH remained same. These reaction mixtures were incubated at 37° C for 30 min and the absorbance was measured at 517 nm. Per cent Radical Scavenging Activity (RSA) upon sample treatment was determined by comparison with a methanol treated control. All the determinations were performed in triplicates. Ascorbic acid was used as positive control. The per cent RSA was calculated using the formula:

$$\text{RSA \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Reducing Power Assay

Reducing power estimation was carried out as described previously by Nagulendran et al (2007) with slight modifications. 0.75 ml of methanolic plant extract solution (1 mg/ ml) was mixed with 0.75 ml of 0.2 M phosphate buffer (pH 6.6) and 0.75 ml of 1% potassium ferricyanide and incubated at 50° C for 20 min. Then, 0.75 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.5 ml of the supernatant solution was mixed with 1.5 ml of distilled water and 0.5 ml of 0.1% FeCl₃. Absorbance was measured at 700 nm in UV-Visible Spectrophotometer using phosphate buffer as blank and butylated hydroxyl toluene (BHT) as standard. The experiments were performed in triplicates.

Antibacterial activity

The ethyl acetate and methanol extracts of plant were dissolved to prepare a stock solution of 50 mg/ ml. The extracts were tested for antibacterial activity with slight modifications (Singh et al., 2009) against pathogenic Gram-positive (*Staphylococcus aureus* MTCC 7443, *Bacillus subtilis* MTCC 121) and Gram-negative (*Escherichia coli* MTCC 7410, *Salmonella typhi* MTCC 733) bacteria. Streptomycin (1 mg/ ml) was used as standard antibacterial agent. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5x10⁸ cfu/ mL. 20 ml of nutrient agar media was poured into each Petri plate, allowed to solidify under aseptic condition and plates were swabbed with 50 µl inocula of the test bacteria and kept for 15 min for adsorption. Sterile discs of 6 mm diameter were loaded with a 10, 20, 30 and 40 µl volume of ethyl acetate and

methanol extracts (50 mg/ ml) were seeded on agar plates. All the plates were incubated at 37° C for 24 h. Antibacterial activity of ethyl acetate and methanol extracts was evaluated by measuring the zone of growth inhibition against the test organisms. The medium with appropriate solvent was used as a negative control whereas streptomycin 10 µg/ disc used as positive control. The experiments were performed in triplicates.

Statistics

The values are mean ± SD of triplicate determinations. The data were analysed by ANOVA followed by Tukey's HSD test for significant differences using SPSS 11.0 computer software. IC₅₀ values were calculated by Boltzmann's dose response analysis using Origin 6.1 computer software.

Results and Discussion

Phytochemical Screening

The solvent extracts of *T. cuneatus* revealed the presence of secondary metabolites such as reducing compounds, alkaloids, flavonoids, saponins, tannins, cardiac glycosides, glycosides, sterols, terpenoids and carbohydrates (Table 1). The results also revealed that, anthraquinones, proteins and amino acids were absent in all the solvent extracts of the plant.

Determination of Total Phenolics

The total phenolic content in the methanol extract of *T. cuneatus* was determined as Gallic Acid Equivalent (GAE). The extract showed concentration dependent increase in phenolic content. Tested methanol extract had good phenolic content of 33 µg of GAE in 100 µg of plant extract.

Antioxidant activity by DPPH assay

The antioxidant activity of the methanol extract measured as free radical scavenging activity is presented in Fig. 1. The leaf extract of *T. cuneatus* revealed a considerable antioxidant activity (IC₅₀ = 102 µg/ ml) in DPPH method. The results also showed that the activity was dose dependent which increased with increase in the concentration of the extract (Fig. 1) which was similar to that of standard. Ascorbic acid showed 50% inhibition at 40 µg/ ml.

Reducing Power Assay

The reducing power ability of *T. cuneatus* extract was compared with standard BHT. An increase in absorbance at 700 nm indicated the reducing power of the extract. The methanolic extract of the plant showed promising amount of reducing power ability which reflected its antioxidant potential. Reducing ability of the methanolic extract of the tested plant increased with respect to increase in the concentration (Fig. 2).

Antibacterial activity

The antibacterial activity of ethyl acetate and methanol leaf extracts at different concentrations of *T. cuneatus* against some of the tested Gram positive and Gram negative pathogenic bacteria are presented in Table. 2. Among the different concentrations tested, extracts treated at 2 mg/ disc showed broad spectrum activity against different test pathogens, but no activity was observed at other concentrations. *B. subtilis* was highly susceptible showing 12.53 mm and 15.13 mm activity to ethyl acetate and methanol extracts, respectively followed by *S. typhi*, *S. aureus* and *E. coli*. The positive control streptomycin offered a maximum of 29.66 mm activity against *B. subtilis* and a minimum of 24.93 mm activity against *E. coli*.

Table.1 Qualitative Phytochemical Analysis of *T. cuneatus* leaf extracts

Secondary Metabolites	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Reducing Compounds	--	++	--	++
Saponins				
a. Foam Test	--	--	++	++
Alkaloids				
a. Wagner's Test	++	++	++	++
b. Dragendorff's Test	--	--	++	++
c. Mayer's Test	--	--	--	++
d. Hager's Test	++	--	--	++
Tannins				
a. Ferric chloride test	--	--	--	++
b. Gelatin Test	--	--	--	--
Sterols				
a. Libermann-Burchard's Test	++	--	--	++
Terpenoids				
a. Libermann-Burchard's Test	--	++	++	--
Anthraquinones				
Flavonoids				
a. Ferric Chloride Test	--	--	--	++
b. Alkaline reagent Test	--	--	--	++
Cardiac glycosides				
a. Keller-Kilani Test	++	++	++	++
Glycosides				
a. Borntrager's Test	--	--	--	--
b. Baljet Test	++	++	++	++
Proteins and Amino acids				
a. Ninhydrin Test	--	--	--	--
b. Biuret Test	--	--	--	--
Carbohydrates				
a. Benedict's Test	--	--	--	--
b. Fehling's Test	--	++	++	++

++ : Present; -- : Absent

Table.2 Antibacterial activity of *T. cuneatus* leaf extracts against test bacterial species

Extract	Concentration	Zone of inhibition (mm)			
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>
Ethyl acetate	0.5 mg/ disc	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	1 mg/ disc	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	1.5 mg/ disc	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2 mg/ disc	10.80±0.80	12.53±0.50	10.43±0.58	11.60±0.52
Methanol	0.5 mg/ disc	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	1 mg/ disc	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	1.5 mg/ disc	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2 mg/ disc	13.46±0.50	15.13±0.41	12.2±0.72	12.43±0.40
Solvent Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Streptomycin		27.50±0.50	29.66±0.61	24.93±0.90	26.50±0.50

Values are mean inhibition zone (mm) ± S.D of three replicates

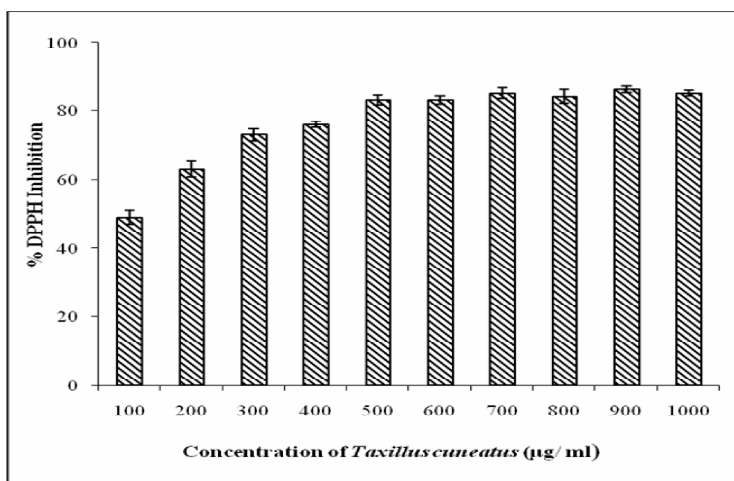


Fig.1 Antioxidant activity by DPPH method of methanolic extract of *T. cuneatus* at different concentrations. Values are mean ± S.D. of three replicates

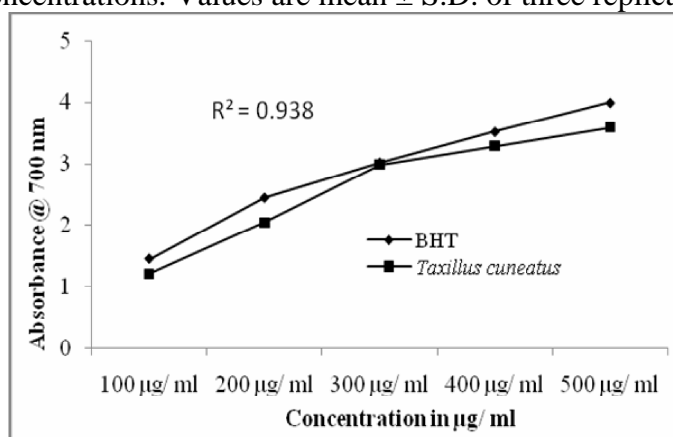


Fig.2 Reducing power of *T. cuneatus* methanolic extract at different concentrations compared with BHT

About 70-80% of the world population is dependent on herbal medicine especially in the developing countries as they have good affinity towards human body without any harmful effects and nearly 21,000 plants are used for this purpose as reported by World Health Organization (WHO). The medicinal value in plants is due to some chemical elements which are responsible for physiological functions in the human body (Liu, 2003). These chemical elements are called phytochemicals (alkaloids, tannins, flavonoids, saponins, sterols, triterpenes and many other compounds) which are found in the plants as product of plant metabolism or synthesized for defence purposes and they are either toxic or useful to human body (Usman and Osuji, 2007). Species of Loranthaceae consists of rich source of phytochemical constituents and are said to have potential medicinal properties including anti-hypertensive, anti-obesity, anti-inflammation, antioxidant, immunomodulatory effects, antiproliferative, anti-cancer, neuroprotective, hypolipidemic effects, treatment of atherosclerosis and also prevent the renal toxic effects (Alleykutty et al., 1993; Osadebe et al., 2004; Pattanayak and Sunita, 2008; Chiu, 1996; Huang et al., 2008; Wang et al., 2008; Zhang et al., 2011; Deng et al., 2011; Lee et al., 2012; Liu et al., 2012; Zhang et al., 2013).

Taxillus spp. are known to possess many medicinal properties and used in traditional medicines. In the present investigation *Taxillus cuneatus* was subjected for determination of phytochemical constituents using different types of solvents. The extracts revealed the presence of secondary metabolites such as flavonoids, saponins, alkaloids, tannins, reducing compounds, sterols, tri-terpenes

and absence of anthraquinones. The presence of these secondary metabolites are known to have therapeutic activity against several diseases and therefore could suggest its traditional use for the treatment of various illness (Yousuf et al., 2012). Earlier studies have reported that flavonoids have antibacterial property as they have the capability to associate with soluble proteins and bacterial cell walls (Doss et al., 2011). These flavonoids also have antioxidant property as they inhibit oxidative and hydrolytic enzymes, have impact on radical scavenging, anti-inflammatory and anti-cancerous activity (Liu et al., 2008; Alsabri et al., 2013). Similarly saponins are reported to act adversely on bacteria and fungi so they are reported to have antimicrobial (Rohit et al., 2012), anticancerous and many health benefits (Shi et al., 2004), while alkaloids are used for their antiparasitic, antioxidant, anticancerous and antimicrobial activity (Alsabri et al., 2013) and tannins are reported to have antimicrobial, antidiarrhoeal, anti-inflammatory, antioxidant activities and have astringent property (Killedar and More, 2010).

Plant phenolic compounds are main contributors of antioxidant activity and are also responsible for anti-inflammatory, antiviral and anticancerous and antimicrobial activities (Yang et al., 2013). The present investigation revealed that the extract of *T. cuneatus* has good phenolic content. Similar results are obtained by Murali et al (2011) and Puneetha et al (2013) wherein the extracts of mistletoes showed total phenol content. Free radicals could play an important role in the degenerative or pathological processes of a range of serious diseases (Chin-Yuan et al., 2007). Many plants are exploited for their antioxidant activity to replace the synthetic one as they have no side effects

and used for the treatment of many dangerous diseases such as cancer, diabetes, stroke, atherosclerosis and Alzheimer's disease (Mosquera et al., 2007). The DPPH radical scavenging activity is an extensively used method to estimate the antioxidant activity and the present study on methanolic extract of *T. cuneatus* showed an enhanced antioxidant activity with increase in the concentration of the extract. The higher radical scavenging activity of *T. cuneatus* seems to be directly correlated with its total phenolic content as it may play an important role in its antioxidative effect. The results were in line with Deng et al (2011), where the ethanolic extracts of *T. liquidambaricola* showed Antioxidant, analgesic, and anti-inflammatory activities. Another important indicator of antioxidant activity is reducing power of compounds which are electron donors and have reductones and perform as primary and secondary antioxidants (Li and Lin, 2010). Reducing power of phytochemical compounds is actively dependent to the antioxidant activity (Yildirim et al., 2001). Similarly, the methanolic extract of *T. cuneatus* showed both antioxidant as well as reducing power ability.

Now-a-days many microbes causing various diseases have become resistance to several old and new pharmaceuticals and researchers are exploring traditional plants for their medicinal properties. There are number of reports on antibacterial activities from plant source (Abdel-Hameed et al., 2012; Yang et al., 2013). Similarly in the current investigation, the crude extracts of the *T. cuneatus* were tested to check its potency against pathogenic bacteria. The results showed varied degree of antibacterial activity with respect to the pathogen tested, solvent extract and its concentration. Likewise,

there are reports on extracts of mistletoes used in antimicrobial activity (Osadebe and Ukwueze, 2004; Pattanayaka and Sunita, 2008; Efuntoye et al., 2010).

The present investigation has thrown a light on the availability of various sources of secondary metabolites present in *T. cuneatus* and its effect on antioxidant and antibacterial activities. Further, the extracts of *T. cuneatus* are subjected for purification of compounds which may act as an alternative for the present synthetic compounds used as pharmaceuticals.

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