The Pharma Innovation Journal 2018; 7(5): 602-606

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(5): 602-606

www.thepharmajournal.com
Received: 24-03-2018
Accepted: 25-04-2018

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Phytochemical and antioxidant evaluation of leaves extracts of *Semecarpus anacardium* L

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Abstract
The present study was covered with leaves extracts of *Semecarpus anacardium*. The extract was prepared with two solvent i.e. water and ethanol screening of phytochemicals was performed with reported method. Both leaves extracts of *Semecarpus anacardium* showed reaction with reagent and reaction was indicated as positive (+) and negative (-). The quantitative estimation of phytochemicals showed moderate result. The antioxidant activity was performed with DPPH method,it showed considerable antioxidant activity.The maximum scavenging(36.70%) activity was shown by ethanolic extract and minimum scavenging (22.21%) activity was shown by aqueous extract of leaves.

Keywords: Leaves, extracts, *Semecarpus anacardium*, phytochemicals, antioxidant activity

Introduction
Plant is a source of large amount of medicinal compounds to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are naturally originated and natural products play an important role in drug development in pharmaceutical industry. (Baker *et al* 1995) [7].
Semecarpus anacardium L. is dry deciduous medicinally important plant, belong to the family Anacardiaceae. It is distributed in Himalaya and sub Himalayan region of India. *Semecarpus anacardium* L. is highly valued for being caustic, astringent, antirheumatic, vesicant and used in anorexia, cough, asthma, indigestion, ulcer piles and various nervous diseases (Chandra, 1989) [11]. Indian knowledge of herbal medicine is gaining widespread acceptance in ayurveda all medicines are prepared from plants, weather it may be raw plant material or refined. (Farnswort and, Soejarto 1999) [14]. As per ayurveda *Semecarpus anacardium* L. is also named as Bhallataka, since ancient times it is used as household remedy on different diseases. It is commonly used as all over India. The nut of *Semecarpus anacardium* L. are used for extraction of oil. The oil of nut is used as mark on cloth for the purpose of identification, hence it is known as marking nut and it is also called as dhobinut. These oil is used as medicine from ancient time and it's also used for waterproofing timber, paint, and medicine. So these nuts is called as Kaldhan told tribal peoples.
Semecarpus anacardium L. is dry deciduous medicinally important plant, belonging to the family Anacardiaceae. It is distributed in Himalaya and sub Himalayan region of India. *Semecarpus anacardium* L. is highly valued for being caustic, astringent, antirheumatic, vesicant and used in anorexia, cough, asthma, indigestion, ulcer piles and various nervous diseases (Chandra, 1989) [11].

Material and Methods
Sample Collection
The leaves of *Semecarpus anacardium* L. were collected from farm region (19˚ 04' 05 N 077^0 14' 49 E) in Nanded district of Maharashtra. The entire twig of *S. anacardium* L. was pressed in wooden card board for herbarium preparation. The pressed plant was then transferred on the standard herbarium sheet and the plant identification was confirmed by Prof. R. M. Mulani and the identified herbarium sheet is preserved in herbarium depository at School of Life Sciences in Swami Ramanand Teerth Marathwada University, Nanded.

Shade dried: Leaves were washed in running tap water for removing of dust particle and foreign particles and shade dried for 8 days.

Preparation of extract: Shade dried leaves were used for preparation of powder with electric blender. These powder were stored in plastic container for further use. Around 25 gm powder used for extraction in 250 ml aqueous and ethanolic solvents Vijayalakshmi *et al.,* (2012) [43]. The extraction was done by Soxhlet extraction techniques till dark colouration of the solvent and discolouration of powder extract. The solvents were evaporated to complete dryness by rotavator and stored in eppendorf's tube at 4 ˚C for further use Hassan *et al.,* (2014) [18]; Das *et al.,* (2014) [13].

Qualitative phytochemical screening

The leaves of *S. anacardium* (L.) were extracted with aqueous and ethanolic solvent. The various qualitative tests were undertaken for detection and identification of primary and secondary metabolites using various method suggested by Gomathi *et.al,* (2013) [16], Santanu *et al.* (2011) [39], Jerald and Jerald (2007) [22].

Quantitative Estimation of phytochemicals

Phytochemicals were estimated by using following reported method- alkaloid (Obadoni and Ochuko, 2001) [31, 10], flavonoid content using (Chang *et al.,* 2002, Boyer, 2003) [12], phenolic content **(**Aiyegroro and Okoh, 2010, Thimmaiah 2004) [2, 42], tannin content with (Saxena *et al.*,2013) [40] **t**otal saponin content by (Obadoni and Ochuko, 2001) [31], and terpenoid was estimated with (Indumathi *et al* 2014) [20]. Estimation of protein and carbohydrate was carried with Biuret and anthrone method (Thimmaiah 2004) [42].

Determination of alkaloids (Obadoni and Ochuko, 2001) [31].

Crude leaves extracts of 5 g of was weight into 250ml beaker. 200ml of 20% acetic acid was added and covered the beaker with aluminum wrapper while stand for 4 hr. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate. The precipitate was collected by filtration and weigh.

Total flavonoid content (Chang *et al.*, 2002, Boyer, 2003) [12, 10].

The total flavonoid in the crude extracts was measured using the aluminum chloride colorimetric method. To 1 ml of plant extract or standard of different concentrations 3 ml methanol was added. Addition of 2 ml of 10 % aluminum chloride. Addition of 0.2 ml potassium acetate (1M), 5.6 ml of distilled water was added. Then the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 510 nm using UV spectrophotometer against a blank. Standard curve was prepared using quercetin by dissolving it in methanol followed by serial dilution to 100 to 1000 µg/ml. Flavonoid content of extracts was determined using standard graph.

Total phenolic content (Aiyegroro and Okoh 2010, Thimmaiah 2004) [2, 42].

The 2.5 ml of 10% Folin-Ciocalteu reagent was added, 2ml of 2% solution of Na_2CO_3 was added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 750nm. Gallic acid was used as standard (1mg/ml). The results was determined from the standard curve and was expressed as gallic acid equivalent (mg/ml of extracted compound) determined from the standard curve and expressed.

Total tannin content (Saxena *et al.*, 2013) [40].

Accurately weight 0.5g of the leaves extracts was transferred to a conical flask. Added 75ml distilled water then heated the flask gently and boil for 30 min. Centrifuged at 2,000 rpm for 20 min and collected the supernatant in 100 ml volumetric flask and made up the volume. Transferred 1 ml of the sample extract to a 100ml volumetric flask containing 75 ml water. Added 5ml of Folin-Denis reagent in 10 ml of sodium carbonate solution was added and dilute to 100 ml with water and shaken well. Read the absorbance at 700 nm after 30 min. (Folin-Dennis reagent- Mixture of phosphomolybdate and phosphotungatate).

Total saponin content (Obadoni and Ochuko, 2001) [31].

For the saponins determination, 5 gm of each plant samples was weighed and was dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The filtrate and the residues was re -extracted with another 100 ml of 20% ethanol. The combined extracts was reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples was dried in the oven to a constant weight. The saponins contents was calculated in percentage.

Estimation of total protein and carbohydrate was determined using reported standard graph method (Thimmaiah, 2004, Sadashivam, and Manickam, 2008) [42, 37].

Antioxidant activity

DPPH radical scavenging assay was carried out as reported method with slight modification (Adedapole *et al* 2009, Roberts *et al* 2006) [1, 5]. Briefly 1ml of test solution (plant extract) was added to equal quantity of 1mMol/L solution of DPPH in ethanol. Then solution kept in dark places for 20 min incubation period at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1mMol/L) was measured as reference compound. Percent scavenging activity was calculated from control using the following formula.

% Scavenging activity =1- (Absorbance of sample÷ Absorbance of control) × 100

Results

The leaves extracts of *S. anacardium* shown positive and negative reaction with the reagents. The observed reaction was indicated as positive and negative as shown in table no. 1.

Table 1: Preliminary qualitative phytochemical screening of aqueous (Aq.) and ethanolic. (Et.) leaves extracts of *Semecarpus anacardium* L.

Sr. no.	Name of Secondary metabolites	Name of test	Leaves aqueous extract	Leaves ethanolic extract
1	Alkaloids	Hager's	+	+
		Wagner's	+	+
		Mayer's	+	+
2	Carbohydrates	Anthron	-	-
		Fehling's	-	-
		Molisch's	+	+
3	Proteins	Biuret's	+	+
4	Flavonoids	Shinoda's	-	-
5	Glycosides	Molisch's	+	+
6	Triterpenoids	Liebermann- Burchard's	+	+
7	Resins	Resin	-	-
8	Saponins	Saponin	+	+
9	Steroids	Liebermann- Burchard's	-	-
		Salkowski reaction	-	-
10	Tannins	Tannin's	+	+
11	Starch	Starch	-	-

Where: (Aq. E) = Aq = aqueous, E. = extract
(Et. E.) = Et = Ethanolic, E. = extract
+ = Present - = Absent

The leaves extracts of *S. anacardium* shown moderate quantity of phytochemicals. The leaves extracts showed variation in amount of phytochemicals as indicated in table. 2.

Table 2: Estimation of different phytochemicals in leaves extracts of *Semecarpus anacardium* L.

Sr. no.	Name of Phytochemicals	Conc.	L. AQ. E.	L. Et. E.
1	Alkaloids	mg/g	1.971	2.406
2	Saponins	mg/g	1.298	1.983
3	Terpenoids	mg/g	1.201	1.432
4	Proteins	µg/ml	0.642	0.763
5	Carbohydrates	µg/ml	0.572	0.561
6	Flavanoids	µg/ml	0.424	0.535
7	Phenol	µg/ml	0.676	0.697
8	Tannins	µg/ml	2.983	2.948

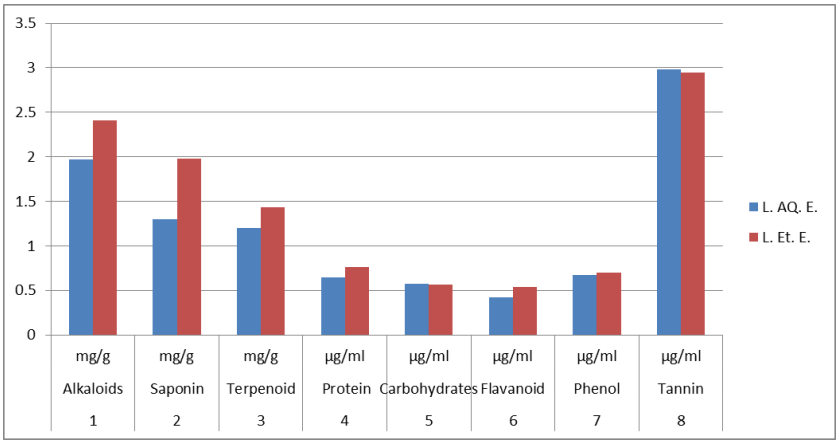


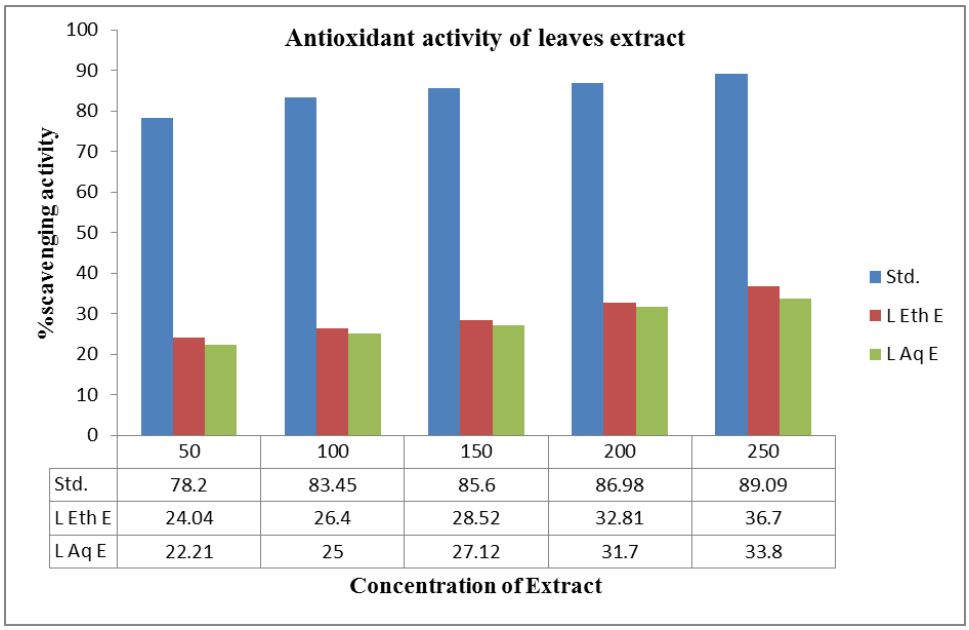
Fig 1: Estimation of phytochemicals in leaves extracts of *Semecarpus anacardium*.

L= Leaves, Et= Ethanolic, Aq.= aqueous E= extract.
The leaves extracts of *S. anacardium* shown moderate scavenging activity of all extracts. The leaves extract showed variation in antioxidant activity as observed in table no. 03.

Table 3: Antioxidant activity leaves extracts of *Semecarpus anacardium* L.

Sr no.	Conc. (µg/ml)	Standard Ascorbic acid		Ethanolic extract(mg/ml)		Aqueous Extract (mg/ml)	
		Absorbance of Ascorbic acid	% scavenging activity	Absorbance of sample	% scavenging activity	Absorbance of sample	% scavenging activity
1	50	0.062	78.20	0.216	24.04	0.221	22.21
2	100	0.047	83.45	0.207	26.40	0.219	25.00
3	150	0.041	85.60	0.203	28.52	0.207	27.12
4	200	0.037	86.98	0.191	32.81	0.194	31.70
5	250	0.031	89.09	0.180	36.70	0.188	33.80

Absorbance of control = 0.284



	50	100	150	200	250
Std.	78.2	83.45	85.6	86.98	89.09
L Eth E	24.04	26.4	28.52	32.81	36.7
L Aq E	22.21	25	27.12	31.7	33.8

Fig 2: Antioxidant activity leaves extracts of *Semecarpus anacardium* L. L= Leaves, Et= Ethanolic, Aq.= aqueous E= extract.

Discussion

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, carbohydrates, proteins, flavonoids, alkaloids and phenols were present in the samples. The work carried with the aqueous and ethanolic leaves extracts of *S. anacardium* which shows the phytochemicals i.e proteins, carbohydrates, phenols, flavonoids, tannins. It was investigated that alkaloid, saponin and terpenoid measured in high amount.

In recent research studies terpenoids, phlobatannins, flavonoids, alkaloids and reducing sugars were absent in *Luffa cylindrica* L., while the previous research studies showed that flavonoids and alkaloids were present in it (Said *et al* 2002) [38]. The phytochemical analysis of showed the presence of terpenoids, reducing sugars and flavonoids. While the previous studies showed that the flavonoids were present (Imran *et al* 2010) [19], the researchers found the same result about the flavonoids and they did not study the remaining phytochemicals in *Acacia nilotica*. The previous research work showed that flavonoids were present in methanolic extract and petroleum ether extracts of *Ficus palmate* (Balakrishnan and Sharma 2013) [8].

The DPPH method was evidently introduced nearly 50 years ago (Blois 1958) [9] and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity**.** Antioxidant activity of the antioxidants is concerning with those compounds capable of protecting the organism system against the potential harmful effect of oxidative stress (Fernández-agulló 2013) [15]. In the present study the effect of different extracts of *S. anacardium* on DPPH scavenging was increasing with the increase in the concentration of the extracts from 50-250 µg/ml and it is thought to be due to their hydrogen donating ability. It has been observed that the extracts prepared in high polar solvents exhibited strong activities which indicates that varieties of polyphenols, and flavanoids trapped may play important role in these activities.

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