ISSN: 0975-833X

Available online at http://www.journalcra.com

SPECIAL ISSUE

International Journal of Current Research
Vol.3, Issue, 6, pp.423-428, June, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PHYTOCONSTITUENTS, PROXIMATE AND MINERAL COMPOSITION OF *Semecarpus anacardium* L. AN ETHNOMEDICINAL PLANT

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ARTICLE INFO

Article History:
Received 3th April, 2011
Received in revised form
17th May, 2011
Accepted 21st June, 2011
Published online 26nd June 2011

Key words:
Semecarpus anacardium,
Secondary metabolites,
Proximate Analysis,
Mineral Composition,
IR, NMR.

ABSTRACT

Semecarpus anacardium L. (Family: *Anacardiaceae*) is a deciduous tree. It is well known for its medicinal properties and used as highly potent Ayurvedic medicine. The fruit is reported to be astringent, antirheumatic, carminative and vesicant. The oil obtained from the nut is used in rheumatic pain. The plant is having high ash content and low moisture content. Phytochemical composition of various extracts of plant parts and minerals were investigated. The results showed presence of secondary constituents comprising of alkaloids (3.18, 1.33 and 2.63 mg/100gm), flavonoids (2.12, 1.12 and 1.78 mg/100g) and phenols (3.22, 1.81 and 2.43mg/100gm) in roots, stems and leaves respectively. The plant is good source of minerals like Sulphur (S), Calcium (Ca), Magnesium (Mg), Phosphorus (P) and Iron (Fe). The IR and NMR spectroscopic analysis of petroleum extract revealed the presence of different functional groups.

INTRODUCTION

The knowledge of chemical constituents of plant would be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1966). Medicinal plants have been receiving great attention worldwide by the researchers because of their safe utility. The curative properties of medicinal parts are mainly due to chemical substances of different composition which occur as secondary metabolites (Karthikayan *et al*., 2009). Medicinal plants form a large group of economically important plants which provide the basic raw material for indigenous pharmaceutical (Aiyelaagbe *et al.,* 2001). The family *Anacardiaceae* contains 700 species distributed among 60 genera. Members of family are cultivated throughout the world for their edible fruits and medicinal compounds. It is a dicotyledonous plant largely trees or shrubs with gummy, milky or resinous juice. It is moderate sized tree distributed in the sub Himalayan tracts. It is distributed in Brahmapuri, Shendewahi and Katol in Marathwada (Almeida, 1996). The plant is scattered in Melghat forest and is medicinally used by villagers (Dhore, 2002). The nuts are used for variety of disorders in Ayurveda. It has been used therapeutically in neurological disorder, ulcers (Kurup, *et al*., 1979), corns (Raghunathan and Mitra, 1982) leprosy, leucoderma (Sivarajan and Indira, 1994).

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Proximate and Nutrient analysis of medicinal plants plays a crucial role in assessing their nutrient significance (Pandey *et al.,* 2006). A close perusal of literature shows that extensive work is being carried out on Nut as compared to other plant parts of *S. anacardium*, though the plant has unique place in Ayurvedic and Siddha medicine. Hence, the present work was designed with an aim to analyze the proximate, minerals and secondary metabolites present in various plant parts. Also, to evaluate the chemical groups in the crude extract using IR and NMR spectroscopic analysis.

MATERIALS AND METHODS

Plant Collection and Identification

The research material was collected from the Melghat forest and Chandur Railway, District Amravati and Kinhiraaja, District Washim during August to October. It was identified in the Department of Botany referring Floras and consulting plant taxonomist. The plant parts leaves, stem and roots were shade and air dried for ten days and milled into fine powder with the aid of an electrical grinder and finally stored in air tight bottles before analysis.

Proximal Analysis

The proximate analysis (Dry matter, moisture content, Ash content, Extractive values) of S. *anacardium* L. was determined by using AOAC (1990). The nitrogen value which is a precursor for protein was determined by MicroKjeldahl Method. The moisture, Ash content and extractive values were determined by (Ahmad, 2005). All the proximate values were reported in percentages (Okwu *et al*., 2004).

Mineral Composition

Dried plant part (5 gm) was taken in Silica crucible and kept in Muffle in Furnace at 400˚C. for 12 hours to obtain ash. About 0.5-1 gm of ash was dissolved in 10 ml of warm 20 % (v/v) HCl in distilled water and the volume was made to 50 ml. The filtrate was used to test the presence of elements. Atomic absorption spectrophotometer (AAS) was used to determine heavy metals concentration. The instrument was calibrated and standardized with different working standards. After making sure that the instrument was properly calibrated and results of standards were in the confidence limit, concentration of metals in each sample was measured individually.

Phytoconstituents

i) Alkaloids: In a 250 ml beaker containing 200 ml of 20% acetic acid in ethanol was taken. To it 5 gm sample was added and covered to stand for 4 h. 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 precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Harborne, 1973 and Obadoni *et al.*, 2001).

ii) Flavonoids: 10 g of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman Filter Paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham *et al.*, 1994).

iii) Phenols: For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipette out into a 50 ml flask and 10 ml of distilled water. 2 ml of ammonium hydroxide solution, 5 ml of concentrated amyl alcohol were added to it. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (Harborne, 1973 and Obadoni *et al,* 2001).

IR Spectroscopy and NMR Spectroscopy:

For the identification of various classes of active chemical constituents the samples were extracted in Petroleum Ether. The extracted product was subjected to NMR spectroscopic analysis. It was carried out in KBr pellets on Perkin Elmer Spectrophotometer (Ramman, 2007).

Statistical Analysis

All the data were expressed as Mean± S.D.

RESULTS AND DISCUSSION

The result analysis showed that the moisture content was low in all parts i.e. below 10, which is an indication that it will not be susceptible to microbial attack (Ogungbenle, 2006). The moisture content of the drug should not be too high thus it could discourage bacterial fungi or yeast growth. Equally important evaluation of crude drug is the ash value and acid insoluble ash value determination (Table 1, 2). The ash content in the plant parts ranged from 10-12% (Table 3) which indicates that high ash content i.e. 10-30% shows presence of all the minerals and marks that the sample could be a better source of essential, valuable and useful minerals needed for good body development. Generally minerals from plant sources are less bio-available than those from animal sources. The extractive values are high in PE and NaOH compared to water (Table 4). These are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Thomas *et al*, 2008). Alcohol has unique feature of dissolving all polar and all non polar constituents (Mukharjee, 2002). The ash value of the drug give an idea of the inorganic composition and other impurities present. The extractive values are used for determination of exhausted or adulterated drug (Singhal *et al.,* 2010).

Elemental analysis reveals the presence of Sulphur (S), Calcium (Ca), Magnesium (Mg), Phosphorus (P) and Iron (Fe) in all parts of plants (Table 6). Minerals play a very important role in the plants. The plant is good source of inorganic minerals like S, Ca, Mg, P and Fe which are nutritionally important. Calcium plays dominant role in the maintaining the strength of stems. Calcium is important in blood clothing, muscle contraction and enzyme metabolism (de *Oliveira et al*., 2001; Freitas *et al*., 2002). The Nitrogen content ranges from 1.2 to 3.2% and is found high in roots and leaves. It is a precursor for amino acids (Table 5). The crude powder of plant parts were analysed for the presence of heavy metals. The results obtained are shown in Table 7. Heavy metals Lead (Pb) and Mercury (Hg) were detected in traces i.e. ppm in leaves, stems and roots from 0.01-0.12 whose toxicity level was very low or negligible. The sample did not exceed the limit given according to the WHO guideline (WHO, 1998). Lead was found to be 0.12 ppm and Mercury 0.07 ppm in roots. In leaves and stem, Pb was found to be 0.01 and 0.02 ppm and Hg was found to be 0.02 and 0.03 in leaves and stem respectivey. It is believed that, presence of lead (Pb) is mainly due to the deposition or adsorption by their external parts. The maximum limit of Pb is 10mg/Kg (Krishnaiah *et al*., 2007) or NMT 20PPM for PB-Pb and Mercury (Shanthi *et al*., 2010)

The amount of secondary metabolites in various parts of plant is presented in Table 8. The alkaloids content was very high in roots 3.18 mg/100gm while leaves contained 2.63 mg/100gm. Flavonoids are high i.e.2.12 mg/100gm, 1.78 mg/100gm in roots and leaves respectively. The content of crude phenols was found high 3.22 mg/100gm in roots and 2.43 mg/100gm in leaves. The roots and leaves are rich in all the three. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic and antispasmodic and bacterial effects (Stray, 1998; Okwu and Okwu 2004). Flavonoids are potent water soluble antioxidant

Table 1. Analysis of Dry Matter

Sr. No.	Plant Parts	Fresh weight (gm)	Dry weight (gm)	Dry content (%)
1	Root	34.82	16.44	47.20±1.32
2	Stem	57.60	24.37	42.30±1.75
3	Leaves	76.86	32.66	42.50±0.70
4	Seeds	60.00	26.64	44.40±0.82

Table 2. Analysis of Moisture Content

Sr.No.	Plant part	Weight of dish (gm)	Fresh weight (gm)	Dry weight (gm)	Moisture content (%)
1	Root	27.00	1.00	0.927	7.3±0.58
2	Stem	27.00	1.00	0.925	7.5±0.05
3	Leaves	27.00	1.00	0.908	9.2± 0.14
4	Seeds	27.00	1.00	0.920	8.0±0.12

Table 3: Analysis of Ash Content

Sr. No.	Plant Parts	Fresh weight (gm)	Dry weight (gm)	Ash content (%)
1	Root	1.00	0.897	10.30±0.12
2	Stem	1.00	0.878	12.20±0.19
3	Leaves	1.00	0.867	13.30±0.16
4	Seeds	1.00	0.868	13.20± 0.05

Table 4. Solubility in various solvents

Solvents	Fresh weight (gm)	Leaves		Stems		Roots		Seeds	
		Dry Wt.	S (%)	Dry Wt.	S (%)	Dry Wt.	S (%)	Dry Wt.	S
Cold Water	1.00	0.872	12.80	0.883	11.70	0.882	11.80	0.888	11.2
Hot Water	1.00	0.724	27.60	0.732	26.80	0.766	24.40	0.784	21.4
P.Ether	1.00	0.442	55.80	0.481	51.90	0.518	48.20	0.528	48.0
1% Hcl	1.00	0.654	34.60	0.668	33.20	0.662	33.80	0.659	34.1
1% NaOH	1.00	0.530	47.00	0.586	41.40	0.533	46.70	0.632	36.8

Table 5. Analysis of Nitrogen

Sr. No.	Plant Parts	Nitrogen Content (%)
1	Root	3.2±0.17
2	Stem	1.8±0.14
3	Leaves	2.4±0.12
4	Seed	1.2±0.09

Table 6. Minerals in various parts of *S. anacardium* L.

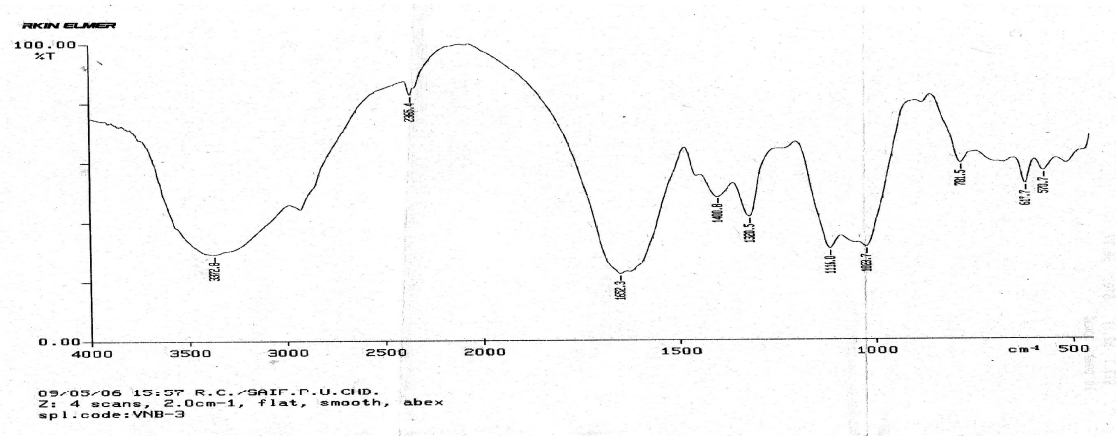
Sr. No.	Plant Parts	Minerals				
		Sulphur	Calcium	Magnesium	Phosphorus	Iron
1	Roots	+	+	+	+	+
2	Stems	+	+	+	+	+
3	Leaves	+	+	+	+	+

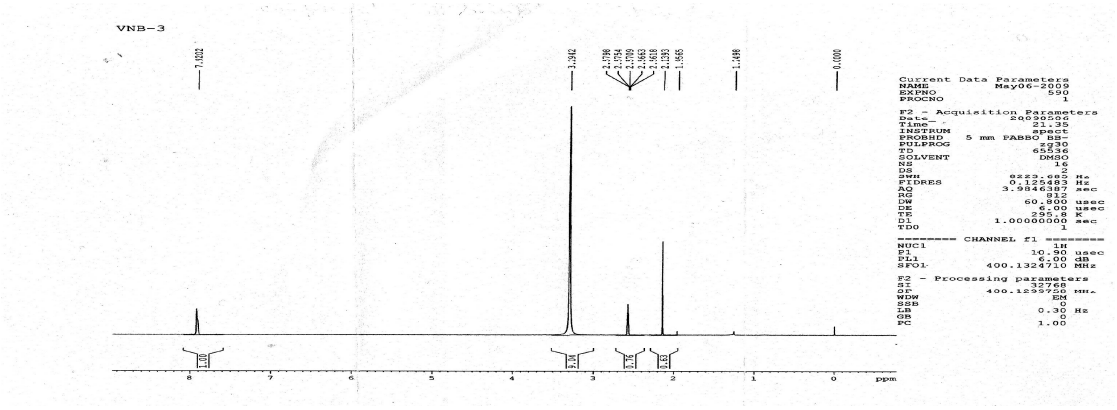
Table 7. Heavy Metals Analysis in various parts of S. *anacardium* L.

Sr. No.	Plant Parts	Heavy Metals (ppm)	
		Pb	Hg
1	Roots	0.12	0.07
2	Stems	0.02	0.03
3	Leaves	0.01	0.02

Table 8. Quantitative Analysis of Secondary Metabolites of *S. anacardium* L.

Sr. No.	Plant Parts	Alkaloids mg/100gm)	Flavonoids (mg/100gm)	Phenols (mg/100gm)
1	Root	3.18±0.08	2.12±0.14	3.22±0.13
2	Stem	1.33±0.09	1.12±0.11	1.81±0.11
3	Leaves	2.63±0.07	1.78±0.25	2.43±0.25



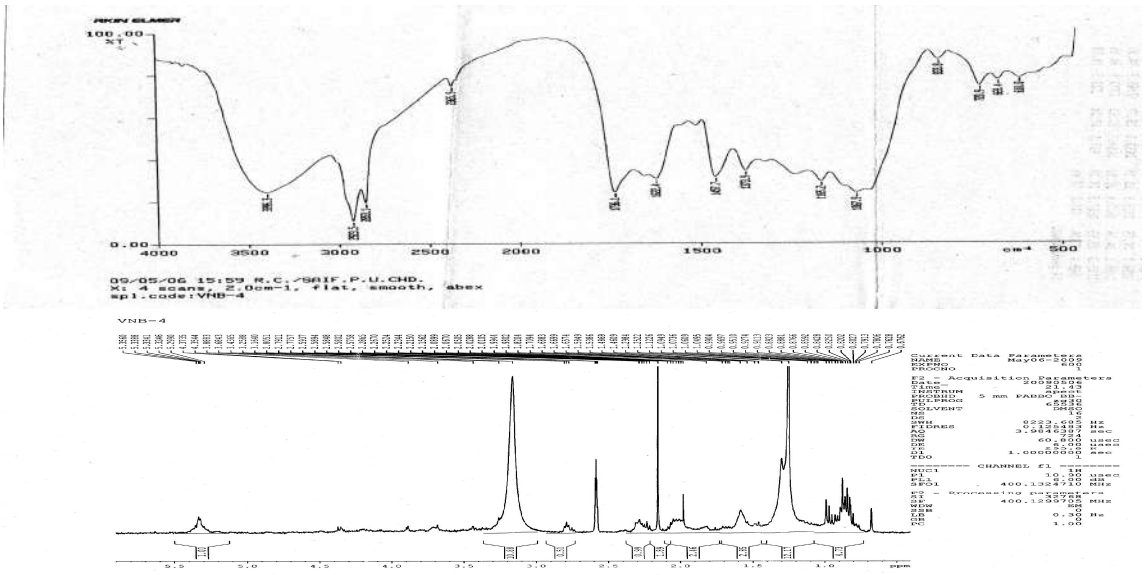


Sr. No	Absorption observed (cm^{-1})	Assignment	Absorption expected (cm^{-1})
1	3372.8	- OH Phenol (Bonded)	3700-3200
2	2365.4	>NH (Imino)	2750-2230
3	1652.3	>C=N (Stretching)	1670-1575
4	1400.8	CH_3 Bending	1450-1375
5	1320.5	Amino Group	1350-1000
6	1116.0	C- O Alcohol	1300-1000
7	1023.7	C- O (Stretching)	1300-1000
8	781.5	Aromatic Stretching (Out of Plane)	900-690
9	617.7	Tri/Di Substituted ring (Stretching)	785-540
10	570.7	C-Br (Stretching)	600-500

Table 9.1. The IR and NMR absorption peaks for root extract of *S. anacardium* L.

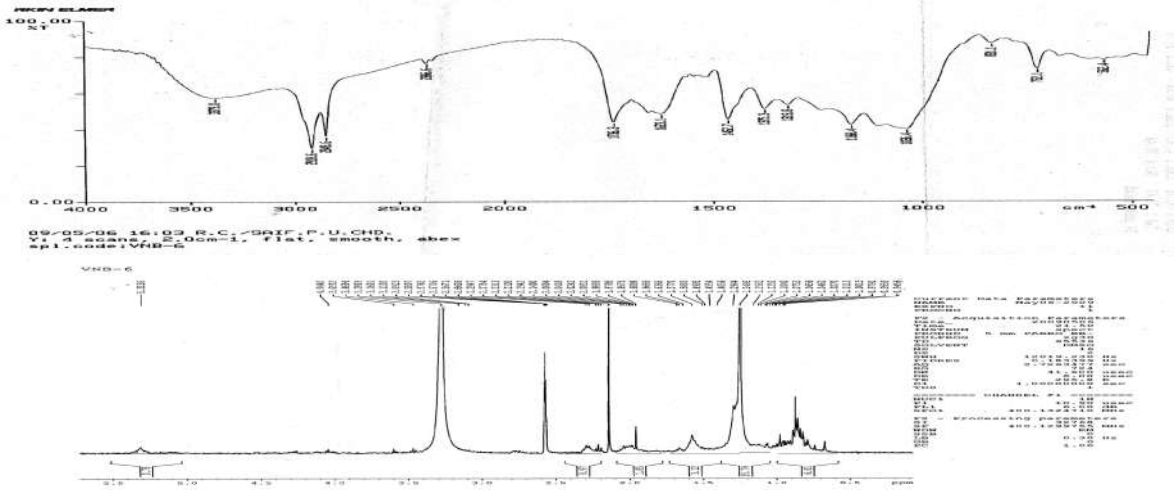
and free radical scavengers which prevent oxidative cell damage have strong anti cancer activity (Salah *et al*., 1995; Del-Rio *et al.,* 1997; Okwu, 2004). Flavonoids indicates anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties (Hodek *et al*., 2002). The presence of phenols in plant indicates that the plant could act as anticlotting agent, antioxidant, immune enhancers and hormone modulator as our spectrum shows the phenolic group (Duke, 1992). The presence of phenolic compounds indicates that they also have antimicrobial activity. This agreed with the finding of (Mohanta, *et al.,* 2007) who reported that aqueous extract fraction (AQE) and organic solvent (PE) extracts showed the inhibitory activity against *Staphylococcus aureus* (10mm) and *Shigella flexneri* (16mm) at 100 mg/ml respectively. While chloroform extract showed inhibition against *Bacillus licheniformis, Vibrio cholera* and *Pseudomonas aeroginosa*. The presence of secondary metabolites has contributed to its medicinal value as well as physiological activity in plant (Sofowora, 1993).

In IR absorption spectroscopy, mid infrared light (4000-2000cm^{-1}) is energetic enough to excite molecular vibrations to higher energy levels. Plant powder clearly shows –OH phenolic stretching at 3372.8 cm^{-1}, 3396.3 cm^{-1} and 3379.0 cm^{-1} in roots, stems and leaves. The peak at 2923.5 cm^{-1} and 2849.6 cm^{-1} >CH_3 grouping (SP_3 Carbon) in stem and leaves which enhances the potency of drug. The peak at 2365.4 cm^{-1} in roots showed the presence of >NH (Imino) group. 2365.9 cm^{-1} and 2366.6 cm^{-1} gave –NH grouping. 1320.5 $cm^{-1}$and 1067.9 cm^{-1} in root and stem showed the peak due to amino group. The peak due to the aromatic stretching 781.5 cm^{-1} , 833.8 cm^{-1} and 831.1 cm^{-1} in roots stem and leaves



Sr. No	Absorption observed (cm^{-1})	Assignment	Absorption expected (cm^{-1})
1	3396.3	- OH Phenol (Bonded)	3700-3300
2	2923.5	$>CH_3$ (SP_3 Carbon)	2930-2850
3	2853.1	$>CH_3$ (SP_3 Carbon)	2930-2850
4	2365.9	-NH Grouping	1670-1600
5	1736.1	-Ester –CO- O-	1750-1730
6	1622.4	>C=C< grouping	1600-1500
7	1457.7	NO_2Grouping	1550-1350
8	1373.9	NO_2 Grouping	1550-1350
9	1165.2	Ether /Alcohol Grouping	1300-1000
10	1067.9	Amino Group	1350-1000
11	833.8	Aromatic Stretching (Out of plane)	900-690
12	669.4	C-Cl Streching	785-540
13	610.0	Tri/Di Substituted ring (Stretching)	785-540

Table 9.2: The IR and NMR absorption peaks for stem extract



Sr. No	Absorption observed (cm^{-1})	Assignment	Absorption expected (cm^{-1})
1	3379.0	- OH Phenol (Bonded)	3700-3300
2	2918.6	-CH_3Grouping	2960-2870
3	2849.6	>CH3 (SP_3Carbon)	2930-2850
4	2366.6	- NH Grouping	2750-2250
5	1736.3	Ester – CO-O	1750-1730
6	1623.1	Aromatic ketonic stretching -	1670-1600
7	1462.7	NO_2 Grouping	1550-1350
8	1375.3	CH_3 Bending	1450-1375
9	1319.8	Sulphoxide Grouping	1350-1140
10	1168.4	Sulphoxide Grouping	1350-1140
11	1036.4	-C=N Stretching	1350-1000
12	831.1	Aromatic Stretching (Out of plane)	900-690
13	722.1	Tri/Di Substituted ring (Stretching)	785-540
14	562.4	C-Cl Stretching	600-500

Table 9.3: The IR and NMR absorption peaks for leaves extract

showed the signals due to Ar-H proton at δ 7.9202 ppm. The signal at δ 3.2942 ppm is due to amino group Ar-NH protons. The signal at δ 2.5618 - δ 2.5798 ppm is due to ($-CH_3$) proton. This spectrum showed the signals due to Ar-H proton at δ 7.7369 ppm. The signal at δ 5.3560- δ 5.2990 ppm is due to amino group Ar-NH protons. The signal at δ 3.2588-2.7757 ppm is due to ($-CH_2$) proton. The signal at δ 1.2984 - δ 1.2522 ppm is due to (-CH3) proton. The signal at δ 0.9904 –δ 0.001 ppm is due to moisture in DMSO- d_6. This spectrum showed the signals due to amino group proton at δ 4.0440-δ 3.2803 ppm. The signal at δ 2.5807-2.5626 and δ 2.2947- δ 2.064 ppm is due to $-CH_2$ proton. The signal at δ 1.2944-1.2493 ppm is due to ($-CH_3$) proton.

The $-CH_3$ group is electron donating group which is responsible to increase the potency in drugs action. The spectroscopic study is carried out for the first time for various plant parts. The wavelengths of IR bands are characteristics of specific types of chemical Bonds. Such type of spectrum was obtained for pure Amoxilline, Ampicillin. The structure of Amoxicillin and Ampicillin drug nucleus shows the same type of functional group as present in plant parts. Thus it can complement for the drugs property. This is for the first time such work is carried out in *Semecarpus anacardium* L. Therefore, it can be concluded that *Semecarpus anacardium* L. Plant is rich in secondary metabolites and minerals composition can be a potential source of useful therapeutic drugs. Attempt is needed to isolate these active phytochemical agents from the plant extracts for pharmaceutical applications in future.

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