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Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES OF *SEMECARPUS ANACARDIUM* (LINN.F.) LEAVES

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ABSTRACT

Semecarpus anacardium Linn.f. belongs to family Anacardiaceae. Literature survey showed that no work has been done on leaf of plant, hence it was selected to study its pharmacognostic and phytochemical properties. This study includes preparation of 14 different polar and nonpolar extracts by soxhlet extraction for detailed analysis. Physicochemical studies showed constant and definite values which will help in the correct identification of the plant. The preliminary phytochemical studies showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides, hexose sugars, diterpenes, mucilages and gums. Further fluorescent analysis of different soxhlet extracts and leaf powder provide additional support for the qualitative chemical analysis findings. The results suggest that the leaves of the plant having phytochemical properties may be used for curing various ailments.

Keywords: Fluorescent analysis, Pharmacognostic, Phytochemical, *Semecarpus anacardium* Linn.f., Soxhlet extraction.

INTRODUCTION

Plants provide not only food, but also useful medicaments to many of our diseases. Adulteration and substitution is very common in any crude drug market. Hence correct identification of medicinal plant is essential. *Semecarpus anacardium* belong to family Anacardiaceae. It has been valued in traditional system of medication for possessing variety of therapeutic properties. A literature survey of the related species shows that lot of work has been done on the fruit, nut and seeds[1-6], however, no work has been carried out on the leaf of the plant. The main aim of our present study is to investigate the pharmacognostic and phytochemical properties of *Semecarpus anacardium* leaves.

MATERIALS AND METHODS

Collection and authentification of plant

The leaves of *Semecarpus anacardium* were collected from an open field around Mumbai, Maharashtra. The identification of the plant was done at the Blatter Herbarium, St. Xavier's College, Mumbai. The plant specimen matches with the Blatter Herbarium specimen no.T-472 of S. C. Tavakari. Leaves were shade dried and made into coarse powder with mechanical grinder and then passed through sieve, B.S.S Mesh No.60.

Physicochemical analysis

The physicochemical parameters like total ash, water soluble ash, acid insoluble ash, sulphated ash, loss on drying, water soluble and alcohol soluble extractive values, pH, foaming index, crude fiber content were determined[7-10], the results of which are given in Table no.1.

Preparation of plant extract

The leaf powder of *Semecarpus anacardium* (20 gms) was extracted with 250 ml each of polar and nonpolar solvents by soxhlet extraction for 8 hrs. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were then stored in plastic bottle in refrigerator for preliminary phytochemical analysis. Powdered leaf material was extracted using water, chloroform, toluene, carbon tetra chloride, ethyl acetate, hexane, ethyl alcohol, methanol, acetone, 2-propanol, petroleum ether (60-80°C), 2-butanone, dichloromethane, ethyl ether and was subjected for identification of various plant constituents[11].

Fluorescence Analysis

Fluorescence characteristics of powdered leaf and leaf extract were examined[12]. The observed results are given in the TableNo.2 and 3 respectively.

Phytochemical screening

Extract of *Semecarpus anacardium* leaves were subjected to phytochemical screening. The results obtained in the present investigation are shown in Table No.4. It showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides, hexose sugars, diterpenes, mucilages and gums[13-15].

RESULTS

The mean values of % w/w ± SEM (Standard error of the mean) of total ash, acid insoluble ash, sulphated ash and water soluble ash are tabulated in the Table No.1. The loss on drying at 105°C in leaf was found to be 3.57 ± 0.25. The analytical results showed that total ash value content was 15.2 ± 0.25. Similarly, the amount of acid insoluble matter present in the plant was 4.76 ± 0.21. The water soluble and alcohol soluble extractive values were 3.30 ± 0.26 and 4.16 ± 0.42. The fluorescence characteristics was also studied under U.V. light (254 and 366nm), wherein the powdered leaf sample and leaf extracts showed the visibility of varying colors which are as tabulated in the Table No. 2 and 3.

Alkaloids were present in extracts of toluene, carbon tetrachloride, ethyl alcohol, methanol, 2-butanone, dichloromethane, petroleum ether 60-80°C, hexane and ethyl ether. Saponins were present in water, carbon tetrachloride, ethyl acetate, acetone, dichloromethane extracts. Carbohydrates were suppressed due to presence of secondary metabolites but they were present in some extracts like 2-butanone and dichloromethane. Glycosides were present in water, chloroform, toluene, carbon tetrachloride, ethyl acetate, hexane, ethyl alcohol, methanol, acetone, 2-propanol, petroleum ether 60-80°C, 2-butanone, dichloromethane. Tannins were present in chloroform, toluene, carbon tetrachloride, ethyl acetate, ethyl alcohol, methanol, acetone, 2-propanol, 2-butanone, dichloromethane. Flavonoids were found in chloroform, carbon tetrachloride, ethyl acetate, ethyl alcohol, methanol, acetone, 2-propanol, 2-butanone, petroleum ether 60-80°C, dichloromethane, ethyl ether extracts.

Similarly, steroids showed their presence in chloroform, methanol, petroleum ether 60-80°C, 2-butanone, ethyl ether, carbon tetrachloride, ethyl alcohol, acetone, 2-propanol extracts. Phenols were not present except for one extract i.e. methanol. Proteins biuret and ninhydrin test did not show any positive results but xanthoproteic tests gave positive results for toluene, carbon tetrachloride, methanol, 2-propanol, 2-butanone, dichloromethane. Monosaccharides were not shown except for ethyl alcohol extract. Hexose sugars were present in all the 14 extracts. Diterpenes was confirmed in water, chloroform, ethyl

acetate, ethyl alcohol, methanol, acetone, 2-propanol, petroleum ether 60-80°C, 2-butanone, ethyl ether extracts. Non reducing polysaccharides i.e. starch were present only in ethyl acetate and methanol. Mucilages and gums were found in water, chloroform, ethyl alcohol, methanol, acetone, 2- propanol. The results are tabulated in Table No.4.

Table 1: Physicochemical test of powdered leaves of *Semecarpus anacardium*

S. No.	Parameters	Mean values (% w/w) ± SEM
1.	Total ash	15.2 ± 0.25
2.	Water soluble ash	7.35 ± 0.08
3.	Acid insoluble ash	4.76 ± 0.21
4.	Sulphated ash	13.31 ± 0.11
5.	Water soluble extractive value	3.30 ± 0.26
6.	Alcohol soluble extractive value	4.16 ± 0.42
7.	Moisture content (Loss on Drying)	3.57 ± 0.25
8.	Crude fiber	7.08 ± 0.29
9.	pH	7.03 ± 0.01
10.	Foaming index	Less than 100

Table 2: Fluorescence Analysis of Powdered Leaves of *Semecarpus anacardium*

S. No.	Test	Visible Light	Under U.V. light (254 nm)	Under U.V. light (366 nm)
1.	Powder as such	Brown	Black	Black
2.	Powder + 5% Aqueous NaOH	Dark Brown	Light Green	Black
3.	Powder + Aqueous 60% H_2SO_4	Pale Yellow	Pale Green	Dark Blue
4.	Powder + conc. H_2SO_4	Black	Dark Blue	Black
5.	Powder + conc.HNO_3	Dark Brown	Light Green	Black
6.	Powder + conc. HCl	Light Brown	Light Green	Black
7.	Powder + Glacial Acetic acid	Dark Brown	Light Brown	Light Pink
8.	Powder + 1N NaOH in Methanol	Dark Green	Dark blue	Light Pink
9.	Powder + Ethanol	Dark Green	Dark Blue	Dark Pink

Table 2: Fluorescence Analysis of Powdered Leaves of *Semecarpus anacardium*

S. No.	Test	Visible Light	Under U.V. light (254 nm)	Under U.V. light (366 nm)
10.	Powder + HNO_3 + NH_3 Solution	Light Brown	Light Green	Black
11.	Powder + 50% HNO_3	Dark Brown	Light Green	Black
12.	Powder + Alcoholic KOH	Dark Green	Dark Blue	Dark Pink
13	Powder + 5% KOH	Dark Brown	Light Green	Black
14.	Powder + Ammonia solution 25% v/v	Dark Brown	Black	Black
15.	Powder + 5% Ferric chloride	Dark Brown	Light Green	Black
16.	Powder + Picric acid	Brown	Black	Black
17.	Powder + Iodine Solution	Dark Brown	Black	Black

Table 3: Fluorescence characteristic of leaf extract of *Semecarpus anacardium.*

S. No.	Leaf Extract	Under ordinary light	Under UV light (254 nm)	Under UV light (366 nm)
1	Water	Light Brown	Light Green	Colourless
2	Chloroform	Light Green	Light Green	Light Pink
3	Toluene	Light Yellow	Light Yellow	Light Yellow
4	Carbon tetra chloride	Light Green	Light Green	Light Pink
5	Ethyl acetate	Light Green	Light Green	Dark Pink
6	Hexane	Light Yellow	Light Yellow	Light Yellow
7	Ethyl alcohol	Light Green	Light Green	Light Pink
8	Methanol	Dark Green	Light Green	Dark Red
9	Acetone	Light Green	Light Green	Light Pink
10	2- propanol	Light Green	Light Green	Light Pink
11	Petroleum ether (60-80°C)	Yellow	Light Green	Light Pink
12	2- butanone	Dark Green	Dark Green	Dark Pink
13	Dichloromethane	Dark Green	Dark Green	Dark Red
14	Ethyl ether	Dark Green	Light Green	Light Pink

Table 4: Qualitative Phytochemical Analysis of Various Extracts of *Semecarpus anacardium* Leaves.

S. No.	Name of theTest Procedure	Observation	Soxhlet Extracts of Leaves													
1.	Alkaloids		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Dragendorff's Test	Orange Red ppt.	-	-	-	+	-	-	-	-	-	-	-	+	-	-
ii	Mayer's Test	Whitish Yellow or Cream coloured ppt.	-	-	-	+	+	-	-	+	-	-	-	-	+	-
iii	Hager's Test	Yellow coloured ppt.	-	-	-	-	-	-	-	+	-	-	-	+	+	-
iv	Wagner's Test	Reddish Brown ppt.	-	-	+	+	-	+	+	+	-	-	+	+	+	+
2.	Saponins		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Foam Test	Foam persists for 10mins.	+	-	-	+	+	-	-	-	+	-	-	-	+	-
3.	Carbohydrates		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Molisch Test	Purple or reddish violet color.	-	-	-	-	-	-	-	-	-	-	-	+	-	-
ii	Fehling's Test	Brick Red ppt.	-	-	-	-	-	-	-	-	-	-	-	-	+	-
iii	Benedict's Test	Red ppt.	-	-	-	-	-	-	-	-	-	-	-	-	+	-
4.	Glycosides		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Legal's Test	Pink to Red color.	+	-	+	+	+	+	-	-	-	-	+	-	+	-
ii	Baljet Test	Yellow to Orange color.	+	+	-	+	-	-	+	+	+	+	-	+	-	-
5.	Tannins		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Lead acetate Test	White ppt.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ii	Ferric chloride Test	Dark Blue or Greenish Black.	-	-	-	-	-	-	-	+	-	-	-	-	-	-
iii	Potassium dichromate Test	Yellow color ppt.	-	+	+	-	-	-	+	+	+	+	-	-	-	-
iv	Gelatin Test	White ppt.	-	-	+	+	+	-	-	+	+	+	-	+	+	-
v	Potassium ferric cyanide Test	Deep red color.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	Flavonoids		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Shinoda's Test	Cherry Red color.	-	-	-	-	+	-	-	+	-	-	-	+	-	-
ii	Alkaline Reagent NaOH Test	Intense Yellow color.	-	+	-	+	+	-	+	+	-	+	-	+	+	+
iii	H_2SO_4 Test	Yellow or Orange color.	-	+	+	+	+	-	+	+	+	+	+	+	-	-
iv	Lead acetate Test	Yellow color ppt.	+	+	-	+	-	-	-	+	+	-	-	+	+	+
7.	Steroids		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Salkowski Test	Bluish red to cherry color in Chloroform layer & Green in acid layer.	-	+	-	-	-	-	-	+	-	-	+	+	-	+
ii	Libermann burchard Test	Brown ring at junction & green or deep red upper layer.	-	+	-	+	-	-	+	-	+	+	+	+	-	+
8.	Phenols		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Ferric chloride Test	Bluish Black color.	-	-	-	-	-	-	-	+	-	-	-	-	-	-
9.	Proteins		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Biuret Test	Pinkish or Purple violet color.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ii	Ninhydrin Test	Blue color.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
iii	Xanthoproteic Test	Orange color.	-	-	+	+	-	-	-	+	-	+	-	+	+	-
10.	Monosaccharide		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Barfoed's Test	Red ppt.	-	-	-	-	-	-	+	-	-	-	-	-	-	-
11.	Hexose Sugars		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Selwinoff's Test for ketohexose like fructose	Red color.	+	-	-	+	-	-	-	+	+	+	-	+	-	-
ii	Tollen's phloroglucinol Test for galactose	Yellow to Red color.	+	+	+	+	+	+	+	+	+	+	+	+	+	-
iii	Cobalt chloride Test	Upper layer Greenish blue & Lower Purplish.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.	Diterpenes		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Copper acetate Test	Emerald Green color.	+	+	-	-	+	-	+	+	+	+	+	+	-	+
13.	Nonreducing Polsaccharides [Starch]		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Iodine Test	Blue color.	-	-	-	-	-	-	-	+	-	-	-	-	-	-
ii	Tannic acid Test	ppt formation.	-	-	-	-	+	-	-	+	-	-	-	-	-	-
14.	Mucilages & Gums		A	B	C	D	E	F	G	H	I	J	K	L	M	N
i	Ruthenium Red Test	Pink color.	+	+	-	-	-	-	+	+	+	+	-	-	-	-

(+) = indicates presence, (-) = indicates absence.

A= water, B= chloroform, C= toluene, D= carbon tetrachloride, E= ethyl acetate, F= hexane, G= ethyl alcohol, H= methanol, I= acetone, J= 2-propanol, K= petroleum ether 60-80° C, L= 2-butanone, M= dichloromethane, N= ethyl ether.

DISCUSSION

New scientific strategies are required for the evaluation of natural products with specific biological activities which requires large screening process. Determination of ash value gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Extractive values were also determined which are primarily useful for the determination of exhausted or adulterated drugs.

A fluorescence characteristic of the dried powdered and soxhlet extracts of leaves of *Semecarpus anacardium* was observed in daylight and UV light. In the near-ultra region of the spectrum (3000 – 4000 A°) some of the phytoconstituents show more or less brilliant coloration when exposed to radiation. Sometimes the amount of ultraviolet light normally present with visible light is sufficient to produce the fluorescence, but often a more powerful source of ultra-violet is necessary, e.g. a mercury vapour lamp[16]. It is often possible to make use of this fluorescence phenomenon for the qualitative examination of herbal drugs. Secondary metabolites from natural sources have been elaborated within living systems and are often perceived as showing more drug-likeness and biological friendliness than totally synthetic molecules making them good candidates for further drug development[17].

Our investigation has found that the plant possesses the biomolecules like alkaloids, saponins, tannins, flavonoids, steroids, glycosides, hexose sugars, diterpenes, mucilages and gums extracts whereas, carbohydrates, phenols, proteins, monosaccharides, starch were found in negligible amount. This indicates that, the presence of other secondary metabolites may have suppressed the activity of proteins and carbohydrates. In addition, the solvent might have also denatured the proteins because of which it is only observed in very less quantity in few extracts. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention[18,19]. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A_2[20]. They also serve as health promoting compound as a results of its anion radicals[21]. Saponins, which are present in plants, have been suggested as possible anti-carcinogens. They possess surface-active characteristics that are due to the amphiphilic nature of their chemical structure. The anticarcinogenic properties of saponins include direct cytotoxicity, immune-modulatory effects, bile acid binding and normalization of carcinogen-induced cell proliferation[22]. The plant extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone[23]. Phenol was not detected in this plant study. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[24].

Thus, the Leaves of *Semecarpus anacardium* may serve as a potential source of bioactive compounds having various medicinal values such as antimicrobial activity, antioxidant activity, etc. Even though this is only a preliminary study, the occurrence of certain phytoconstituents in the leaf of *Semecarpus anacardium* will provide a good concrete base for evaluation of all the phytochemicals functions mentioned above.

CONCLUSION

In the present study, we have identified the biologically active phytochemicals like alkaloids, saponins, tannins, flavonoids, steroids, glycosides, hexose sugars, diterpenes, mucilages and gums present in the 14 soxhlet polar and nonpolar extracts of the leaves of *Semecarpus anacardium*. Further studies are in progress in our laboratory to check the medicinal properties of the leaves of the plant. The present study on preliminary phytochemical and physicochemical analysis of *Semecarpus anacardium* leaf could be used as the diagnostic tool for the standardization of medicinal plant. WHO parameters discussed here, can be considered as the identifying parameters to substantiate and authenticate the drug.

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