PRELIMINARY PHYTOCHEMICAL EVALUATION OF LEAF EXTRACTS OF *CATUNAREGUM SPINOSA* THUNB.

Surabhi Shrivastava[*] and Leelavathi S[2]
[*]Centre for Shridevi Research Foundation (CSRF), Department of Biotechnology, SIET, Tumkur – 572 106, India.
[2]Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore – 570 006, India.
***Email:** Karnatakasurabhi0710@hotmail.com

ABSTRACT

Catunaregum spinosa Thunb is tree distributed in tropical and subtropical regions of the world and about 14 species are found. Present paper deals with preliminary phytochemical evaluation of leaf of *Catunaregum spinosa* Thunb to establish authenticity and possibly to help to distinguish drug from other species. The study includes preparation of different extracts by successive solvent extraction for detailed analysis. Fluorescence analysis of different successive extracts and powder were noted under UV light and normal ordinary light, which signifies there characteristics. Different physicochemical parameters such as, ash value, extractive value, foaming index, hemolytic index, total tannin content and microbial count were carried out as per WHO recommended physicochemical determinations and authentic phytochemical procedures. Preliminary qualitative chemical test for different extract shows the presence of Glycosides, Carbohydrates, Phytosterols/triterpenoids, Saponins, Fixed oils & Fats and phenolic/tannins.

Keywords: *Catunaregum spinosa* Thunb, physicochemical, phytochemical, successive solvent extraction.

INTRODUCTION

Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light modern scientific knowledge[1]. *Catunaregum spinosa* Thunb (Rubiaceae) is an important medicinal plant and popularly known as emetic nut. It is found in waste places & jungles all over India, extending northwest to the Bias river & ascending to outer Himalaya to 4000 ft. Ceylon, Java, & South China. It is carminative, alexiteric, antipyretic; cures abscess, ulcers, inflammations, wounds, tumours, skin diseases[2-4]. It contains triterpenoidal saponins, essential oil, veleric acid, tannins and resin[5]. The present study is designed to explore the preliminary phytochemical and physicochemical analysis of *C spinosa* leaf, which is responsible for its pharmacological properties.

MATERIALS AND METHODS

Plant Material

A survey was conducted during 2007 to 2008 in Biligirirangana Hill of Chamarajanagar District, Karnataka to collect the plant material of *Catunaregum spinosa.* Botanical species were also collected from the forests of Nanjangud area, Mysore district, Karnataka, voucher specimens were collected, identified properly consulting a flora and the same has been deposited in the Department of Botany, University of Mysore, Mysore-06.

Leaves were shed dried and powdered to 40-mesh size. The physicochemical parameters like extractive values, florescence characteristics of powdered leaf and leaf extract, preliminary phyto-profiling and phytochemical analysis, were determined as per WHO guidelines[1]. The average percentage w/w of the ash content and the extractive values were determined. The Fluorescence analysis was carried out according to the reported method[6,7] wherein the color of the powdered leaf and leaf extract were also studied under ordinary and ultra-violet light at 366nm. Powdered leaf material was successively extracted with Petroleum ether, Benzene, Chloroform, Acetone, methanol, ethanol and water in soxhlet apparatus and was subjected for identification of various plant constituents[8,9].

Extraction of plant leaf material

The powdered plant leaf material was subjected to successive solvent extraction taking from polar to non-polar solvents like water, ethanol, methanol, acetone, chloroform, benzene and petroleum ether. 20gms of powdered plant material was subjected to soxhlet extraction for 8 hrs with 250ml of the various solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place for the analysis for the presence of preliminary pytochemicals.

Analysis of primary and secondary metabolites in the extracts of *C. spinosa*

The primary metabolites like; proteins, carbohydrates and fixed oils and fats, were analyzed for their presence as per the standard procedures[7,8]. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolics, tannins volatile oils, terpeniods and glycosides were also assessed in the leaf extracts of *C. spinosa.*

All the data generated from the study were subjected to arithmetic mean with standard deviation for statistical analysis.

RESULTS AND DISCUSSION

All the results generated from the present study are represented in the respective tables. The powdered leaf of *C. spinosa* was subjected to Preliminary physicochemical

and phytochemical analyses which were found to be very promising.

Physicochemical values and Fluorescence characters of the plant powder under ordinary light and UV light (UV 366 nm) were determined and are tabulated in Table 1, 2 and 3(a, b). The determination of ash value was carried out which gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash, sulphated ash and water soluble ash are carried out and results are as tabulated in the Table 1. Extractive values were also determined which are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble extractive values were also determined. The fluorescence characteristics was also studied under ordinary and UV light (366nm), wherein the powdered leaf sample and leaf extracts showed the visibility of varying colors which are as tabulated in the Table no. 3(a) and 3(b). The preliminary phyto–profiling for the leaves extracts of C. spinosa was carried out wherein the consistency was found to be sticky in the non polar to not so polar solvent extracts whereas the polar solvent extracts were found to be non-sticky. The percentage yield w/w of the extracts was also analysed wherein the highest yield was found to be in the ethanolic extract-8.05%. (Table no. 4) The preliminary phytochemical screening revealed the presence of terpenes, phytosterols, phenolic compounds, carbohydrates and saponins (Table 5).

The Plant *C. spinosa* was subjected for Preliminary Phyto-chemical analysis viz; Physico-chemical parameters, primary metabolites like, Protein, Carbohydrate, Fixed oils and fats and secondary metabolites like; alkaloids, flavonoides, saponins, volatile oils, phenols and tannins, glycosides, terpenoides and mucilage were tested wherein, the presence of Carbohydrates was observed in the Acetone, methanol and water extracts respectively.

New scientific strategies for the evaluation of natural products with specific biological activities require the implementation of large screening process. Our investigation has founded that the plant possesses the biomolecules like, Carbohydrates in Acetone, Methanol, ethanol and water extracts whereas, Proteins & amino acids were found in negligible amount. This indicates that, the presence of secondary metabolites may have suppressed the activity of Proteins. In addition, the solvent might have also denatured the proteins because of which it is only observed as very less quantity in aqueous extracts.

Physio-chemical parameters of the leaf extract of *Catunaregum spinosa* are tabulated in Table 1. 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 loss on drying at 105°C in leaf was found to be 5.9 %. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. The analytical results showed that; total ash value content was 6.97 %. Similarly; negligible amount of acid insoluble siliceous matter present in the plant (0.78%) was observed. The water soluble extractive value was indicating the presence of sugar, acids and inorganic compounds and the alcohol soluble extractive values indicate the presence of polar constituents like phenols, steroids, glycosides, flavonoids are represented in the Table 1.

Preliminary phytochemical results showed the presence and absence of certain phytochemicals in the extract. The tests were performed using different organic solvents; Petroleum ether, Benzene, Chloroform, Acetone, Methanol, Ethanol and Aqueous extracts respectively. Phyto-chemical test revealed the presence of triterpene, saponins, flavonoids, polysaccharides, steroids and tannins (Table 5).

The presence of phyto-chemicals in *C. spinosa* extract revealed that, tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer; similar reports were also made by previous Researchers[12,13]. 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[14]. Flavonoids serve as health promoting compound as a results of its anion radicals[15]. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores[10,11,16,17].

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 proposed mechanisms of anticarcinogenic properties of saponins include direct cytotoxicity, immune-modulatory effects, bile acid binding and normalization of carcinogen-induced cell proliferation. However, the anticarcinogenic effects of saponins from commonly consumed plant foods have not been studied. Soybeans are one of the most important sources of dietary saponins. They are the main protein supplier in many vegetarian diets.[18]

The plant extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone[19]. The presence of these phenolic compounds in this plant contributed to their anti-oxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. This plant is used routinely among many tribes in Africa for the treatment of various diseases. Alkaloid 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[20], and their absence in this plant tend to lower the risk of poisoning by the plant.

Thus, *Catunaregum spinosa* containing these compounds may serve as a potential source of bioactive compounds in the treatment of cancer.

Table 1: Physicochemical characterization of leaf of *Catunaregum spinosa*

WHO Parameters	Average values %w/w Leaves
Total ash	6.97
Acid insoluble ash	0.78
Water soluble ash	1.81
Sulphated ash	7.80
Alcohol extractive value	20.93
Water extractive value	9.85
Loss on drying	5.9

Table 2: WHO Parameters for leaves of *Catunaregum spinosa*

WHO parameters	Leaves of Catunaregum spinosa
Hemolytic activity	MeOH ext.:100mg/ml not shows haemolysis Water ext.:11.5
Foaming index	Less then 100
Total tannin content	1.039 %
Total bacterial count	9 x103
Total fungal count	8 x104
E. Coli Salmonella	7 x 103 Absent

Table 3(a): Florescence characteristic of leaf powder of *Catunaregum spinosa*

S. no.	Particulars of the treatment	Under ordinary light	Under UV light (366 nm)
1	Powder as such	Dark green	Brick red
2	Powder + 1N NaOH (aqueous)	Green	Brick red
3	Powder +1N NaOH (alcoholic)	Dark green	Reddish green
4	Powder + 1N HCL	Blackish green	Chocolate brown
5	Powder + H_2SO_4 (1:1)	Green	Brown
6	Powder + HNO3 (1:1)	Yellow	Orange
7	Powder + Ammonia	Greenish yellow	Greenish yellow
8	Powder + Iodine	Dark brown	Brown
9	Powder + 5% FeCl3	Dark-yellowish brown	Dark brown
10	Powder + Acetic acid	Light green	Orange

Table 3(b): Florescence characteristic of leaf extract of *Catunaregum spinosa*

S. No.	Extract	Under ordinary light	Under UV light (366 nm)
1	Petroleum ether (40-60°C)	Green	Yellowish green
2	Benzene	Dark green	Red
3	Chloroform	Dark green	Red
4	Acetone	Dark green	Red
5	Methanol	Dark green	Deep red
6	Ethanol	Dark green	Brown
7	Water	Brownish green	Blackish brown

Table 4: Preliminary phyto-profile for leaves of *Catunaregum spinosa*

S. No.	Solvent used	Color	Consistency	% Yield w/w
1	Petroleum ether (40-60°C)	Green	Sticky	1.4
2	Benzene	Dark green	Sticky	0.6
3	Chloroform	Dark green	Sticky	0.18
4	Acetone	Dark green	Sticky	0.99
5	Methanol	Dark green	Nonsticky	7.07
6	Ethanol	Dark green	Nonsticky	8.05
7	Water	Brown	Nonsticky	1.5

Table 5: Phytochemical analysis of different extracts of *Catunaregum spinosa* leaves

S.No	Name of the Test	Procedure	Observation	♦P	♣B	♥C	♦A	♪E	●M	Aq
1	Alkaloids	Drug + Dragondroffs reagent	Orange color	–	–	–	–	–	–	–
		Mayer's reagent	White ppt.	–	–	–	–	–	–	–
		Hager's reagent	Yellow ppt.	–	–	–	–	–	–	–
2	Glycosides	Anthrone + H2SO4+ Heat	Purple or green	–	–	–	+	+	+	–
3	Carbohydrates	Drug + Molishs reagent+ conc.H2SO4	Purple color	–	–	–	+	+	+	+
		Fehling's solution A&B	Brick red color	–	–	–	+	+	+	+
4	Phytosterols /triterpenoids	Liebermann Test	Bluish green	+	+	+	+	+	+	–
		Salkowski Test	Red & fluorescent	+	+	+	+	+	+	–
		Noller's test	Pink color	+	+	+	+	+	+	–
5	Proteins & Amino acids	Biuret test	Violet color	–	–	–	–	–	–	+
		Xanthoprotein test	Orange color	–	–	–	–	–	–	+
		Millon's reagent test	White ppt	–	–	–	–	–	–	+
		Lead acetate test	White ppt	–	–	–	–	–	–	+
		Ninhydrin test		–	–	–	–	–	–	+
6	Saponins	Drug + water + shaking	Formation of honey comb like froth	–	–	–	–	+	+	+
7	Flavonoids	Shinodaw's Test	Red color	—	–	—	+	+	+	—
		Zn-HCl acid reduction Test	Magenta color	–	—	–	+	+	+	—
8	Fixed oils & Fats	Spot test	Stains appear after drying	+	–	–	–	–	–	–
9	Gums/Mucilage	Drug + water	No thickening of the substance	–	–	–	–	–	–	–
10	Volatile oil			+	–	–	–	–	–	–
11	Phenolics /Tannins	Fecl3	Intense color	–	–	–	+	+	+	+
		Drug + lead acetate + water	Formation of white ppt							

CONCLUSION

The present study on preliminary pytochemical and physicochemical evaluation of *Catunaregum spinosa* leaf could be used as the diagnostic tool for the standardization of medicinal plant. WHO parameters as per WHO guidelines discussed here, can be considered as the identifying parameters to substantiate and authenticate the drug.

Acknowledgement: The authors sincerely thank the authority of Centre for Shridevi Research Foundation – CSRF, SIET, Tumkur for permitting me to carry-out Research work.

REFERENCES

1. World Health Organization, Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1998.
2. Nadkarni AK and Nadkarni KM, The Indian Materia Medica, Popular Prakashan, Bombay, 1976, 1047-1048.
3. Kirtikar KR and Basu BD, Indian Medicinal Plants, Panni office, Bhuwaneswari Ashrama, Allahabad, 1991, 648-652.
4. Agrawal SS and Singh VK, Immunomodulators - A review of studies on Indian medicinal plants and synthetic peptides, Part- 1, Medicinal plants, Proc. Indian Natl. Sci. Acad., 65 Suppl B, 1999, 179-204.
5. Wealth of India, A dictionary of Indian Raw material and Industrial Products. National Institute of Science Communication (C.S.I.R.), New Delhi, Vol. V, 1959, 360-364.
6. Chase CR and Pratt RJ, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, J. Am. Pharmacol. Assoc., 38, 1949, 32.
7. Kokate CK, Practical Pharmacognosy, 1st ed., Vallabh Prakashan, New Delhi, 1986 b, 111.
8. Harborne JB, Methods of extraction and isolation. In: Phytochemical Methods. Chapman & Hall, London, 1998, 60-66.
9. Brain KR and Turner TD, The Practical Evaluation of Phytopharmaceuticals, Wright-Scientechnica. Bristol, 1975b, 36-45. Source of support: Nil, Conflict of interest: None Declared1213
10. Mathekga ADM: Antimicrobial Activity of Helichrysum Species and the Isolation of a New Phloroglucinol from Helichrysum caespititium. PhD thesis. University of Pretoria, Chemistry Department; 2001
11. Lourens ACU, Reddy D, Baser KHC, Viljoen AM, Van Vuuren SF: In vitro Biological activity and essential oil composition of four indigenous South African Helichrysum species. J Ethnopharmacol 2004, 95:253-58.
12. Ruch RJ, Cheng SJ, Klaunig JE: Prevention of cytotoxicity and inhibition of Intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogens 1989, 10:1003-1008.
13. Motar MLR, Thomas G, Barbosa Fillo JM: Effects of Anacardium occidentale stem bark extract on in vivo inflammatory models. J Ethnopharm 1985, 95(2-3):139-142.

14. Li H, Wang Z, Liu Y: Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yao-Cai 2003, 26(6):444-448.

15. Hausteen B: Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharm 1983, 32:1141-1148.

16. Fergusion LR: Role of plant polyphenols in genomic stability. Mutat Res 2001, 475:89-111.

17. Grierson DS, Afolayan AJ: Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape. S Afr J Ethnopharmacol 1999, 66:103-106.

18. Rao AV, Sung MK. Saponins as anticarcinogens. J Nutr (1995) 125:717S–24S.

19. Okwu DE: Evaluation of the chemical composition of medicinal plants belonging to Euphorbiaceae. Pak Vet J 2001, 14:160-162.

20. Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA: Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 1994, 368 (6473): 753-756.

****