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Ethnobotanical, phytochemical and Fourier Transform Infrared Spectrophotometer (FTIR) studies of *Catunaregam spinosa* (Thunb.) Tirven

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ABSTRACT

Catunaregam spinosa **(**Thunb.) (Rubiaceae), commonly called as Gedhphal and madanaphala in Ayurveda. Various ethno medicinal aspects are present in folk literature and new scientific documentation. It's reported that, *Catunaregam spinosa* used as abortifacient also in diarrhoea, anthelmintic, antipyretic and dysentery like problems. Ehno botanical and phytochemical studies were taken out for searching its chemical active ingredient with various referenced scientific methods. The plant material collected from Toranmal forest of Nandurbar Dist. After complete processing and practicing for their proximate analysis, phytochemical test for different secondary metabolites presence, UV–Vis *Spectral analysis* and *Fourier Transform Infrared Spectrophotometer (FTIR)* were taken with different solvent system. As a result it's found that difference in value of leaves, stem bark and root barks parts for proximate analysis for both fresh and dry material has been recorded. It also found that in diffident solvent system presence of secondary metabolites such as phenol, alkaloids etc has been recorded in different plant parts. Lastly with the help of UV and FTIR spectral peak values and functional groups obtained for the leaf extract from Methanol, Chloroform and Ethyl acetate has been recorded. The complete experimental result concludes that Ethnomedicinal information form folk and ancient literature reveals the therapeutic efficiency of *Catunaregum which* need to farther elaboration in future works to detect active ingredient for particular disease.

KEY WORDS: Catunaregum, Ethnomedicine, Ethnobotany, Phytochmistery, Fourier Transform Infrared Spectrophotometer (FTIR), Secondary Metabolites.

1. INTRODUCTION

Catunaregam spinosa **(**Thunb.) Belongs to the family Rubiaceae. The genus *Catunaregum* (*Xeramphis Rafin.*) Consist of about 10 species, out of which two are in India. In Ayurveda it is known as madanaphala. Its formulations and preparations are Madanadi Lepa, Saindhavadi Taila (Brithat), Patoladiniruha vasti, Jivantyadyamvasana vasi and Guduchyadiniruha vasti. Parts of plant like stem and root bark importance in treatment of diarrhoea, dysentery and as well as abortifacient, anthelmintic and antipyretic. Medicinal value also considered to be sedative and hypoglycemic used in case of stomach ache as first aid remedy, roots are used in the treatment of epilepsy, eye ache and urinary infection, fruit of *C. spinosa* is used as fish poison, emetic and the leaves are used in Pulmonary Infections (Warrier, 1999, Santapu, 1973 and Sharma, 2000).

Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light the modern and current scientific knowledge (WHO, 1998). *Catunaregam spinosa* Thunb. (Rubiaceae) is shows alexiteric and antipyretic activity with also significant in skin diseases, inflammations, ulcers, wounds and tumours like diseases (Agrawal, 1999). It contains secondary metabolites like triterpenoidal saponins, tannins, essential oil, resin and veleric acid (CSIR, 1959). The current study is relative to explore presence of phytochemical and secondary metabolites profile analysis of *C spinosa* leaf, Stem and Root. Those are responsible cause for its pharmacological properties. The various analyses carried out in *P. zeylanica* have shown varied and appreciable results similar for *C. spinosa*. *Catunaregam spinosa* were used in our study to evaluate preliminary phytochemical analysis of different plant parts. Phytoconstituents reported like D-mannitol, 1-keto-3α-hydroxy Oleanane, saponins, scopoletin, iridiodal and glycosides, such as garenoside, randioside and geniposide. Then steroids and fatty acid such as caprlic acid, capric acid, tauric acid, myristic acid has been report from different workers (Sharma, 2000; Agrawal, 1999; Iyengar, 1976).

2. MATERIALS AND METHODS

Field Work: *Caturnaregam spinosa* plant was collected from the forests of Toranmal of Shahda Tahsil of Nandurbar Disterct (21.840213° N, 74.456583° E). With collection ethno-botanical importance's from local tribal people's farther cross with exiting literature of ethno-medicine. Plant sample were collected for lab work and also recorded, geographical location for revisit in feature collection of plant as per need.

Laboratory work: Taxonomical identification of plant sample was carried out by the help of flora like Flora of Presidency of Bombay, Flora of Dhule and Nandurbar District and recorded taxonomical characters**.**

Proximate Analysis: It was taken for identification for moisture, Dry matter and Ash content.

Moisture content: The moisture of the sample was lost by volatilization caused by heat. Moisture of the material

was determined by following the method by AOAC (Association of Official Analytical Chemists) 1990. Dishes were washed with detergents and then were dried at 105°C in oven for overnight. Then dishes were removed from oven and then kept in dessicator for cooling and weights. For said procedure estimation was carried out in triplicate and mean values of both were recorded to calculate the moisture content using the following relationship. 2 gm sample of different plant part was taken separately in dishes and placed in oven at 105°C overnight. The moisture content in plant parts was calculated by using the following formulae:-

$$\text{Moisture content (\%)} = \frac{(\text{Weight of fresh sample} - \text{Weight of dry sample})}{\text{Weight of fresh sample}} \text{x } 100$$

Dry matter Content: The dry matter of the sample represents the amount of material left after the complete removal of moisture from it. Dry matters of the sample were determined by following the method by AOAC (Association of Official Analytical Chemists) 1990. Dishes was washed with detergents and then were dried on 105 ^{0}C in oven for overnight. Then dishes were removed from oven and farther than kept in dessicator for cooling. For said procedure estimation was carried out in triplicate and the mean values of both were recorded for calculate the dry matter Content.

2 gm sample were taken in dishes and placed in oven at 105 ^{0} C overnight in Oven. The moisture was calculated with using following formulae:-

$$Dry\ matter\ (\%) = \frac{(Weight\ of\ dish\ +\ Weight\ of\ dried\ sample) - Weight\ of\ dish}{Weight\ of\ sample\ before\ drying}\ x\ 100$$

Ash Content: Ash value was determined by following the method of AOAC (1990). For this crucible were kept in muffle furnace on 600 ^{0}C for 1h. Then they were transferred crucible from furnace to desiccator and then cooled it to room temperature. Sample then weighed quickly to prevent external moisture absorption. Two gram dry sample of plant part was taken in tared silica crucible and placed in muffle furnace on 600 ^{0}C for 6h. Then crucible was transferred to desiccator and allowed for cooling at room temperature, crucible was transferred immediately to avoid moisture absorption after processing. The percentage of presence ash content was calculated with using the following formula,

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \text{x } 100$$

Phytochemical studies:

Alkaloids: 0.5ml extract of sample + treated with help of few drops of 1ml 2N HCl +Mayer's reagent / Dragandorf reagent Hager‟s reagent. Orange precipitate Orange color, White ppt. Yellow ppt. (Vaishali, 2013).

Anthraquinone: Few drops of plant part extract boiled with 10% HCl for couple of minutes and cool then + $CHCl_3$ (Chloroform) to filtrate & few drops of NH_3 added and heated. Rose pink color (Yusuf, 2014).

Cardiac glycosides: 0.5ml plant part extract + 1ml distilled water + aqueous solution forms, addition some drops of NaoH for color observed Brown interface, violet ring below and greenish ring at lowest part (Vaishali, 2013, Kangogo, 2014).

Coumarins: 2 ml of extract, 3 ml of 10% NaOH, Appearance of yellow colour indicates presence of coumarins (Dharmendra, 2012).

Flavonoids: 0.5 ml plant part extract + 5-10 drops of dilute HCl + then boiled for few min. Shinodaw‟s Test, Zn-HCl acid reduction Test. Red color Magenta color (Krishnaiah, 2007; Dharmendra, 2012; Yusuf, 2014).

Glycosides: Anthrone + H_2SO_4+ Heat, Purple or green (Dharmendra, 2012; Yusuf, 2014).

Phenols: $FeCl_3$ Sample + lead acetate + water, Intense color Formation of white ppt (Dharmendra, 2012; Yusuf, 2014; Kangogo, 2014).

Reducing sugars: 0.5 ml Plant part extract was dissolved into 5ml of distilled water and filter it + with addition of Fehling's solution A and B boiled for few min. Presence of orange red precipitate indicates positively detection of reducing sugars (Yusuf, 2014).

Saponins: Sample + water + shaking, Formation of honey comb like froth, Presence of froths/foams (Dharmendra, 2012; Yusuf, 2014; Kangogo, 2014).

Steroids: Salkowski's test and Liebermann burchard's test. Presence of dark green color at upper layer and red color in the lower layer indicating steroidal presence (Dharmendra, 2012; Vaishali, 2013; Yusuf, 2014).

Tannin: 0.5ml of aqueous extract + 10% lead acetate few drops, Greenish-black colouration (Vaishali, 2013; Yusuf, 2014; Mohammad, 2014).

Triterpenes: Liebermann Test, Salkowski Test and Nollers test, Bluish green or red fluorescent, Reddish-brown coloration interface (Vaishali, 2013; Yusuf, 2014; Kangogo, 2014).

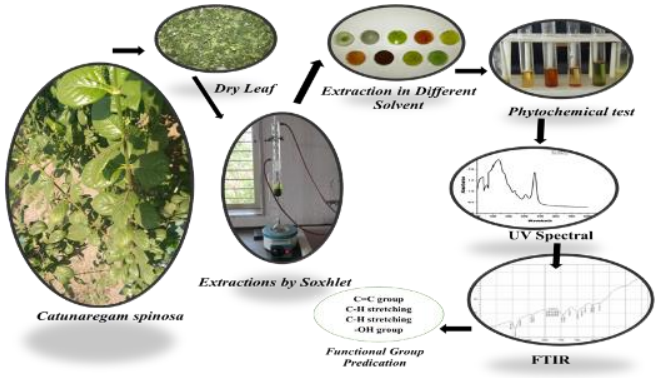


Figure.1. Plant sample and experimental flow chart

UV Visible Spectral Analysis: Extraction of dry leaves was carried out by 5 g of powder for 24 cycle of soxhlet extract. Three solvent were used for extraction viz. Methanol, Ethanol and Chloroform. Different concentrations and dilutions were making for UV analysis in 300nm to 1000nm range for all three samples in replicate (Table 1.).

Table.1. Concentration of Solvent extract for spectral analysis

Concentration	Methanol Extract	Ethanol Extract	Chloroform Extract
20%	200µl/ml	200µl/ml	200µl/ml
40%	400 µl/ml	400 µl/ml	400 µl/ml
60%	600 µl/ml	600 µl/ml	600 µl/ml
80%	800 µl/ml	800 µl/ml	800 µl/ml
100%	Pure extract	Pure extract	Pure extract

Fourier Transform Infrared Spectrophotometer (FTIR): FTIR is the most powerful and applicable tool for identification of chemical bonds (functional groups), and their types present in sample. The wavelength which absorbed light in different variation is characteristic and predictable information for the chemical bond. Which can be seen in the annotated spectrum of FITR. By interpreting the infrared absorption spectrum, the chemical bonds present in molecule can be determined. (Yang, 2002; Martín, 2005; Duraes, 2008). Dried powder of plant sample for different solvents extracts were used in FTIR analysis with 100 mg KBr pellet as encapsulate in sample discs. The powdered plant part sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with the Scanning range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3. RESULT AND DISCUSSIONS

Ethnobotanical Information: Information collected from tribal's and tribal's doctors its foud that, Stem and Root bark used to cure tonsils and neck pain among tribes of satpuda, rarely common practices but effective. Root of this plant and fruits are used to lighten and consequently remove the scars of pimples. Some maids use it as purgative for skin itching in which root bark is processed with cow urine, making past like substance for seven days and applied over to the whole body to cure skin problems (Tribhubana Panda and Rabindra Padhy, 2008).

Therapeutic uses Fruit: It cures disease like ulcers, abscess, inflammation, wounds, and tumours and has antibacterial activity. The pulp of fruit is believed to have anthelmintic properties, abortiffcient contents in folklore remedy (Agrawal, 1999).

Bark: Plant bark is astringent and is given in cases of diarrhoea and dysentery (Chopra, 1956). Stem Bark use for rheumatism by past to applied externally which also relieve pain of bone aches. The aqueous root bark extracts act as insecticide as a bio control agent (Dastur, 1962).

Proximate Analysis:

Table.2. Result for Proximate test

Plant part used	Moisture content	Dry matter Content	Ash Content
Leaves (w/w)	87.50 %	12.50 %	09.50%
Stem Bark (w/w)	46.00 %	54.00 %	17.50%
Root Bark (w/w)	59.50 %	40.50 %	13.50%

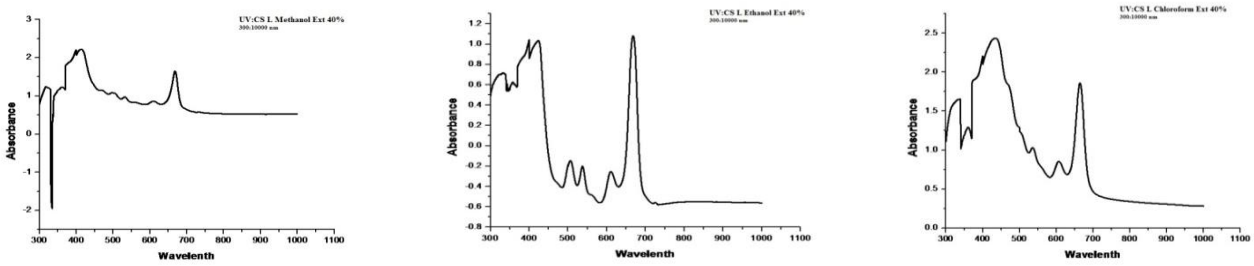
Primary Phytochemistry:

Table.3. Result for Primary Phytochemistry

Sr. No.	Phyto- constituents	*Catunaregam spinosa*																	
		LEAVES						STEM BARK						ROOT BARK					
	Solvent System	W	M	E	Et	C	A	W	M	E	Et	C	A	W	M	E	Et	C	A
1	Alkaloids	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
2	Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

3	**Cardiac glycosides**	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-
4	**Coumarins**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	**Flavonoids**	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
6	**Glycosides**	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
7	**Phenols**	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
8	**Reducing sugars**	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
9	**Saponins**	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-
10	**Steroids**	-	-	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	+
11	**Tannin**	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-
12	**Triterpenes**	+	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
W=Water, M=Methanol, E=Ethanol, Et=Ether, C=Chloroform and A=Acetone																			

UV Visible Spectral Analysis:



Graph.1. UV spectra 40% Conc. for Methanol, Ethanol and Chloroform respectively

FTIR Spectral Analysis: The FTIR spectrum interpretations of leaf in different solvent.

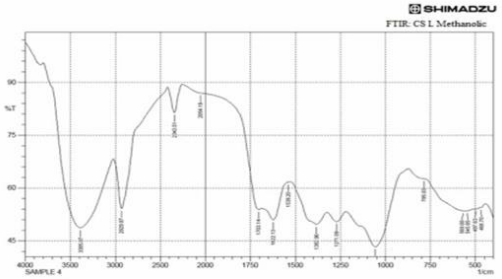
Methanol (ME) extract: The ME extract of *C. spinosa* Leaves showed characteristic absorption bands at 3385 cm^{-1} for a hydroxyl (-OH) group 2929 cm^{-1}, 2343 cm^{-1} (for C-H stretching), 1382 cm^{-1} (for C-H bending), and at 1622 cm^{-1} for C=C group.

Chloroform (CF) extract: For *C. spinosa* Leaves characteristic absorption band were exhibited at 2912 cm^{-1} (for C-H stretching), 1492 cm^{-1} (for C-H bending) for C-H group and at 1710 cm^{-1}, 1718 cm^{-1} for carbonyl groups (C=O) were exhibited by CF extract.

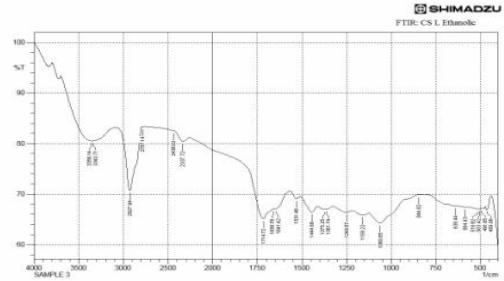
Ethyl acetate (EA) extract: The EA extract of *C. spinosa* Leaves showed the characteristic absorption bands were observed at 2927 cm^{-1} (for C-H stretching), 1444 cm^{-1} (for C-H bending) for C-H group and at 1714 cm^{-1}, for a carbonyl group (C=O).

Table.4. FTIR spectral peak values and functional groups obtained for the leaf extract

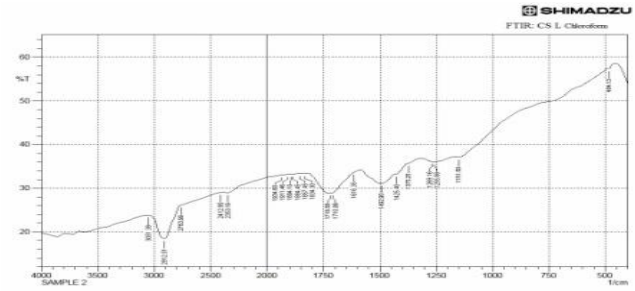
Extracts prepared in	Peak values	Functional groups
Methanol (ME)	1382	C-H bending
	1622	C=C group
	2343	C-H stretching
	2929	C-H stretching
	3385	-OH group
Chloroform (CF)	1492	C-H bending
	1710	C=O carbonyl group
	1718	C=O carbonyl group
	2915	C-H stretching
Ethyl acetate (EA)	1444	C-H bending
	1714	C=O carbonyl group
	2927	C-H stretching



Graph.2. FIIR of *C. spinosa* Leaves in Methanol



Graph.3. FIIR of *C. spinosa* Leaves in Chloroform



Graph.4. FIIR of *C. spinosa* Leaves in Ethyl acetate

4. CONCLUSIONS

The current work from the survey and field observation its find that there is very significance medicinal important of *Catunaregam spinosa* among tribes. As well as it has been found that it has been used for multiple purpose in different area like fruit has an abortiffcient activity (Agrawal, 1999), bark used for diarrhoea and dysentery (Chopra, 1956), sometime root bark past also important in rheumatism, relieve pain of bruises and bone aches during fevers and to disperse abscesses (Dastur, 1962).

Lab and experimental reveals that Moisture content of Leaves (w/w), Stem Bark (w/w) and Root Bark (w/w) are 87.50%, 4600% and 59.50% respectively. Dry matter content of Leaves (w/w), Stem Bark (w/w) and Root Bark (w/w) are 12.50%, 5400% and 40.50% respectively. Ash content of Leaves (w/w), Stem Bark (w/w) and Root Bark (w/w) are 09.50%, 17.50% and 13.50% respectively (Table.2).

For another Parameter in phytochemical test for different primary and secondary metabolites it find that different solvent are effected the positive of result. For leaves, Stem Bark and Root Bark sample it was found that except Anthraquinone, Coumarins and Steroids were not detected among all parameters in six different solvent (Table.3).

UV Visible analysis graphical representation recorded between 300:1000 nm shows sharp peak in Leaf sample in 400 µl/ml of different extract (Graph 1).

FTIR spectral peak values and functional groups obtained in Methanol (ME) are five which showing five fictional group presence in leaf sample such as C-H bending C=C group, C-H stretching, C-H stretching and -OH group. In Chloroform (CF) and Ethyl acetate (EA) of leaf sample fined functional group are four and three respectively. They are C-H bending, C=O carbonyl group, C=O carbonyl group and C-H stretching in CF were C-H bending, C=O carbonyl group and C-H stretching in EA (Table.4).

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