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Research paper

Isolation & characterization of Phytoconstituents from a medicinal plant cocciniagrandis & tinosporacordifolia

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The plants *CoccaniaGrandis*&*Tinosporacordifolia*have been found to be source of medicinal agents based on their use in traditional medicines.In present work the taxonomic identification of collected plant by Mr. P.J.Parmar.In brief preparation of plant material by different techniques such as drying, grinding, sieving.Extraction of plant material by using different solvents ethanol, methanol, hexane and isolation of compound by chromatography has been done.Purification of isolated compounds has been done and characterization of isolated compounds has been done through spectral techniques like IR, NMR, UV, Mass spectrum.UV spectra have shown maximum absorption due to substitution of functional group in compound no (I).The IR spectrum of compounds (I) showed absorption bands characteristics of flavanol indicating the presence of hydroxyl and tinsoporin functionalities at 3449, 1375 and 1079 cm^{-1} respectively, indication of double bond were represented by 1689 cm^{-1}. peak at 1375 cm^{-1} represented C-H bending (Fig. 5.1a) where as NMR spectra (Fig 5.1b) was also characteristic of phytotaxanol, exhibiting the hydroxyl proton signal at δ 5.21as a multiplet, which helped us to characterize this compound as taxaterone, tinsoporin 4A,4B respectively.

Keywords: cocciniagrandis, Tinosporacordifolia, Hepatoprotectiveactivity.

INTRODUCTION

Ancient Chinese, Indian and European discovered origin of medicinal herbs. They have been using them for curative purposes successfully. The records are available in ancient texts. In India itself, there are more than 20000 medicinal plants grown all over the wild forests. Of these, some 60 genus are used immensely in medicinal preparation. Despite their demands today, they are not grown in controlled manner. Rather tribes use them as their livelihood in some belts where they are grown in the wild. Unlike India, in china, the spurts in demand for traditional medicine have made government to allow growth of these plants for further research and development. About 100 units have nearly 600 plant type, grown for their medicinal value.

Herbal medicines are used in Ayurveda, Naturopathy and Homeopathy, tradition and Native American medicine.[1]

The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the medicinal plant plays a major role and constitutes the backbone of traditional medicine.

A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs .The plants that possess

therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants, which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, contain minerals and vitamins, possess medicinal properties.[2]

Chemical Constituents:

Aerial part - Heptacosane, Cephalandrol, β -sitosterol, Alkaloids Cephalandrins A and B.

Fruits- β- Amyrin Acetate, Lupeol, Cucurbitacin B, Taraxerone, Taraxerol, β-carotene, Lycopene, Cryptoxanthin, Xyloglucan, Carotenoids, β-sitosterol, Stigma-7-en-3-one.

Root - Resin, Alkaloids, Starch, Fatty Acids, Carbonic acid, Triterpenoid, SaponinCoccinoside, Flavonoid Glycoside, Lupeol, β-amyrin, β-sitosterol, Taraxerol.

Pharmacological Activities:

Antibacterial: Evaluated the aqueous extractof leaves of Cocciniagrandis for antibacterial activity againstShigellaflexneri NICED, Bacillus subtilis Escherichia coli,Salmnellacholeraesuis, Shigella dysenteries, and Shigellaflexneri,.**Anthelmnintic:** Methanolic extract of Cocciniagrandisposses the anthelmintic activity.

Antioxidant: Moideen evaluated Ethanol extract of root of Cocciniagrandis contain flavonoids which are responsible for antioxidant activity.

Antiulcer: The anti-ulcer activity aqueous extract of leaves of Cocciniagrandis was investigated in pylorus ligation and ethanol induced ulcer models in experimental rats.**Hepatoprotective:**Vadivu evaluated the alcoholic extract of the fruit of cocciniagrandis for Hepatoprotective activity against CCl4- induced Hepatotoxicity in experimental rats, Treatment with 250 mg/kg ethanolic extract of fruit significantly reduced the SGPT, SGOT and bilirubin level.

Anti Inflamantory: Deshpande evaluated the aqueous extracts ofCocciniagrandis leaves and stem for the anti-inflammatoryactivity against formaldehyde Induced paw edema in rats.

Analgesic: Acetic acid induced writhing, Tail immersion and Hot plate models were used to evaluate the analgesic activity.**Antimalarial:** Extract of Cocciniagrandis shows excellent antiplasmodialactivity against the Plasmodiumfalciparum.**Anticancer:** There are a number of vegetables occurred to reduce the risk of cancer. One of them is Cocciniagrandis.

AIM OF WORK

Aim of Present Study:

As we know very well that everything in this world change time by time, since thousands of year the era was of Ayurveda or herbal origin drug. But last few decades it was replaced by allopathic system of medicine, which was fastly accepted world wide, but later due to its lots of

adverse effect again men step down on Ayurveda because of its better therapeutic result and safety profile and now the people are more believing in natural origin drug.

As briefed in the previous sections, the plant have been found to be source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Hence the overall aim of the present work was to study phytochemical characterization of a medicinal plant which is used traditionally in (RAJ.)

The criteria of selection of plant for our work were based on:

A. Known use of the species as herbal remedy, primarily in Rajasthan and also worldwide.

B. The availability of the plant locally in large quantities.

C. Special attention was paid to access plant that how much phytochemical work is not done. Further investigation may give some promising results.

Therefore this plant is having wide scope for detailed Phytochemical (including TLC, IR, NMR, and MS, followed by spectral studies) investigation.

PLAN OF WORK

To fulfill above object in research envisaged the work was to be complete in following manner:

Review of literature

Collection of plant.

Taxonomic and ethno medicinal identification of the collected plant by Mr. P.J. Parmar (Deputy Director of botanical survey of india, near khemkakuaon ,nandan van, Jodhpur-324008)

Preparation of plant material (by drying, grinding, sieving).

Extraction and fractionation.

Preliminary physical and phytochemical study of different extract and fraction.

Qualitative phytochemical analysis.

Quantitative phytochemical analysis.

Isolation and purification of phytoconstituents.

Characterization of isolated phytoconstituents by spectral studied using by IR, NMR, MASS spectral data.

EXPERIMENTAL WORK

Plant Selection: Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethno botanist, ethnopharmacologist, or plant ecologist who identifies the plant of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. On the basis of intensive literature survey; *CoccaniaGrandis*&*Tinosporacordifolia* were selected for present study.

Collection and identification:

Plant was collected from the India, during the months of august and September 2015. Taxonomic and ethno medicinal identification of the collected plant by Mr. P.J. Parmar (Deputy Director of botanical survey of india, near khemkakuaon ,nandan van, Jodhpur-324008)

Preparation of plant material:

The aerial parts of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

Analytical Parameter:

Ash Values:

The residues remaining after incineration is the ash content of the drug. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information regarding its adulteration with inorganic matter. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, and water soluble ash.

Determination of total ash value:

Accurately weighed about 3 gm of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

Determination of acid insoluble ash value:

The ash obtained as directed under total ash value was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water soluble ash value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. All the ash values were calculated and recorded in table (4.1A) & (4.1B).

Extractive Values:

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of Alcohol Soluble Extractive Value:

10gms of the air-dried coarse powder of *CoccaniaGrandis*&*Tinosporacordifolia*were separately macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of

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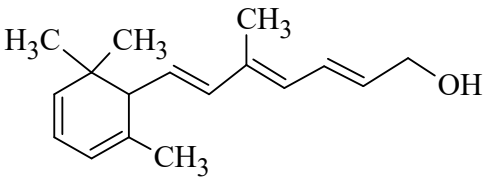
ethanol soluble extractive value was calculated with reference to the air-dried drugs

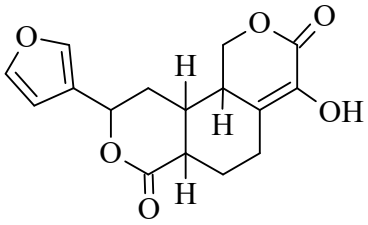
RESULTS AND DISCUSSION

Spectral analysis and structural elucidation:

It was tried to purify the compounds, which were obtained by employing column chromatography (CC) and preparative thin layer chromatography(PTLC), and by re-crystallizing them in different solvents. The compounds were weighed and there melting point and solubility was determined. The identification of a molecule was done through the interpretation of the data obtained from spectroscopic analysis. Tools for vital structure elucidation of natural products are Fourier transform infrared spectroscopy (FTIR) Agilent Cary 630 FTIR spectrometer , mass spectrometer (MS) Agilent 6520 (Q-TOF) , and nuclear magnetic resonance spectroscopy (NMR) Bruker II Avance 400 FT-NMR . With these tools the structures of most natural products can be determined.



2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)hepta-2,4,6-trien-1-ol(COMP-4A)



9-(furan-3-yl)-4-hydroxy-1,5,6,6a,9,10,10a,10b-octahydro-3H,7H-pyrano[3,4-f]isochromene-3,7-dione (COMP-4B)**Compound -4A**

UV spectra have shown maximum absorption due to substitution of functional group in compound no (I).The IR spectrum of compounds (I) showed absorption bands characteristics of flavanol indicating the presence of hydroxyl and tinsoporin functionalities at 3449, 1375 and

Compound (4A,4B)- Results shown in table

IR (KBr) ν_{max} **cm^{-1}**	**^{1}H NMR (in ppm) δ**	**Melting Point range**	**Ultra Violet Wave Length**	**Mass Spectra**
3683 (alcohols, phenols) Stretch 3397 (OH stretch, H-bonded) 1635 (aromatics) 1521 (C-C stetch) 849 (C-H stretch)	0.675 s br (methyl) 1.49 s br (ethyl) 1.6 s br(ethyl) 1.9 s br (Propyl) 2.18d (J= 12.0 Hz) 5.21d (J= 4.0, 2.0 Hz) (ether)	230-232 ^{0}C	max= 259 Absorbance= 0.813	Base Pea207. M+ Peak242.2

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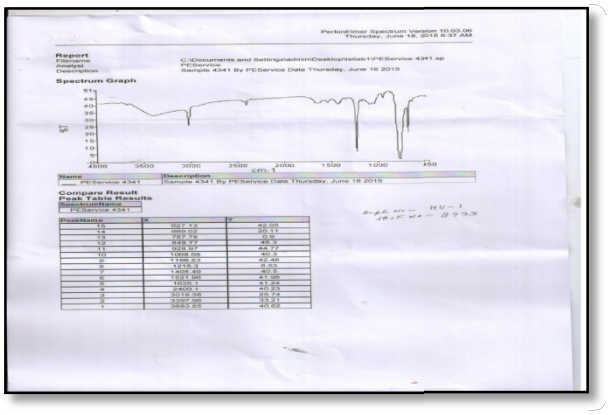

Fig. 1: - IR spectrum of compound no (4A)

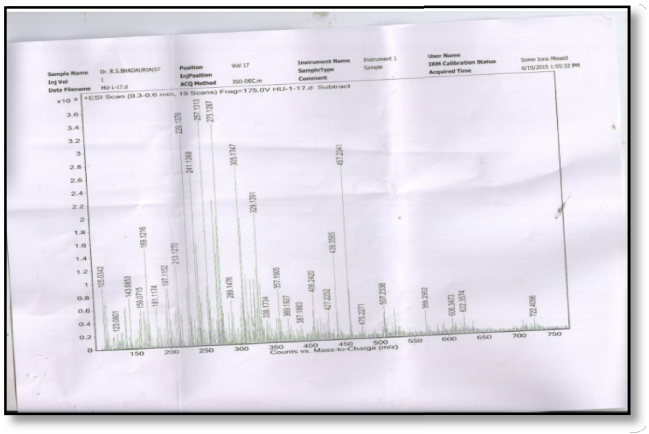

Fig.-2: Mass spectrometry of compound

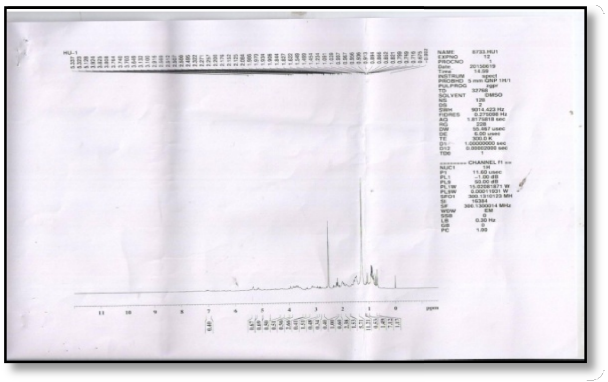

Fig 3:^{1}H-NMR spectra of compound (4A)

1079 cm^{-1} respectively, indication of double bond were represented by 1689 cm^{-1}. peak at 1375 cm^{-1} represented C-H bending (Fig. 5.1a) where as NMR spectra (Fig 5.1b) was also characteristic of phytotaxanol, exhibiting the hydroxyl proton signal at δ 5.21as a multiplet, which helped us to characterize this compound as taxaterone, tinsoporin 4A,4B respectively.

CONCLUSION

The plants *CoccaniaGrandis*&*Tinosporacordifolia*have been found to be source of medicinal agents based on their use in traditional medicines.In present work the taxonomic identification of collected plant by Mr. P.J.Parmar.

In brief preparation of plant material by different techniques such as drying, grinding, sieving.

Extraction of plant material by using different solvents ethanol, methanol, hexane and isolation of compound by chromatography has been done.

Purification of isolated compounds has been done and characterization of isolated compounds has been done through spectral techniques like IR, NMR, UV, Mass spectrum.

UV spectra have shown maximum absorption due to substitution of functional group in compound no (I).The IR spectrum of compounds (I) showed absorption bands characteristics of flavanol indicating the presence of hydroxyl and tinsoporin functionalities at 3449, 1375 and 1079 cm^{-1} respectively, indication of double bond were represented by 1689 cm^{-1}. peak at 1375 cm^{-1} represented C-H bending (Fig. 5.1a) where as NMR spectra (Fig 5.1b) was also characteristic of phytotaxanol, exhibiting the hydroxyl proton signal at δ 5.21as a multiplet, which helped us to characterize this compound as taxaterone, tinsoporin 4A,4B respectively.

Compound no (5A) tested positive for polyphenol by giving dark intensy violet color with ferric chloride reagent [107]. It represent IR spectrum in the regions 3418, 2931, 1733, 1663, 1617, 1456, 1366 and 1257 (Fig 5.1a), indicating the presence of characteristics features for polyphenols

Compound no (5B) tested positive for polyphenols. It presented IR spectra in the regions 3417, 2915, 2847, 1729, 1662, 1460, 1364, and 1259 cm $^{-1}$ (Fig 5.1 a) indicating the presence of characteristics features for polyphenols [108-110]. the ^{1}H NMR of this compound exhibited signals in the range of 6.89-7.97 and 3.4-6.34 ppm (Fig 5.1 b) that belonging to phenolic hydroxyls and D-glucose molecules respectively, signals in the region 6.23-7.97 ppm assigned to Gallic acid protons.

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