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Phytochemical investigation of *Peristrophe bicalyculata* (retz-nees)

B. M. Sharma, S. S. Babaria^{*} *M. M. Science College (Morbi) – 363642 E-mail: brishsharma@gmail.com Received: 25th May, 2011 ; Accepted: 25th July, 2011

ABSTRACT

Since ancient time man has adopted several type of remedies from nature, a review of literature reveals that from tinniest herb to tallest tree or the climber are one or the other way useful in human or animal therapy. A pioneering work was done by our Saints Charak, Shushrut and several others, which was collected in the form of vedas (Rigveda,Ayurveda) Samhita, Nighantu and Aryabhishak. Work was done by several scientists around the world which led to compilation of information with regard to Occurrence, Identification, Chemical constitution, and therapeutic properties of such drugs, particularly used in ayurvedic medicines. Now the great scope is there for the scientist regarding herbal therapeutics, lacs of plants have been investigated and now reinvestigation is carried out for there chemical composition and therapeutic properties as per discovery of different new diseases, Peristrophe bi- calyculatta (Retz-Nees) is reported to be the useful remedy for the treatment of Jaundice, Manorhaegia, T.B, Antiseptic and Anti-venom agent in indigenous system of medicine. We have isolated several constituents but here will be discussing isolation and characterization of Phenolic constituents which were confirmed by folin and coecaltaus reagent and then extracted and its antimicrobial activity was studied comparing it with standard allopathic drug.

INTRODUCTION

A verse from old literature Kashyap Samhita proclaims that the use of drugs derived from plants dates back to antiquity what at present we take may be in the crude or pure form in the treatment of the alleged disorders was for shadowed in the folk remedies of the olden days and are coded in literature by Ayurvedic pioneers like charak, shushrut, Agnivesh Vagbhatt, and several other followers, which was collected in the form of vedas (Rigveda, Ayurveda) Samhita, Nighantu and Aryabhishak.

The level of the concentration of the effective molecule (drug) is low but enough to cure the disease faster by Ayurvedic method of healing. Now the great scope is there for the scientist regarding herbal therapeutics, lakhs of plants have been investigated and now reinvestigation is carried out for there chemical composition (extraction of the active constitution) and therapeutic properties as per discovery of new diseases

Some locally used preparations have been found to surpass the efficiency of the synthetic drug used for the same treatment. Few of the plants are soo much usefull that it can cure certain allopathically incurable diseases. Ayurveda is not a literature but (package of medication), a well developed science, it is a wholesome guide to live a healthy life.

Ayurveda has guided How to stay fit with using natural methods ie pathya, vipathya, ahar, vihar etc these all are written In the form of shlokas, in the literatures Veda, Nighantu, Samhita Aryabhishak by pioneers like Charak, Kashyap, Vagbhat Shushrut Sharangdhar and

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several other Rishis. The goal of life is to die young as late as possible.

At present Life style disease like diabetes, allergies, insomnia, obesity has large impact in human life.

Desire of the aging population to stay young and healthy led to self medication and greater orientation towards preventive health care.

Pharmaceutical Companies switched over to Phytopharmaceuticals because the Ayurvedic remedies have lowest side effects.

Few of the diseases are such that they have no alternative other than ayurveda for epilepsy drug Mangrove (Excoecaria Agllocha)

Turmeric is used for Gastrointestinal System, anti spasmodic and anti flatulent Anti inflamatory Anti Rheumatism Antidiabetic, Anti carcinogenic even what we consider as unwanted i.e. The weeds also has medicinal value like Indian Todophylum has Radio protection properties.Ginger Pipali and Asafoetida have potential to treat Gastric Ulcer. Sikakai has Saponin for hair washing but on reinvestigating it has cytotoxic activity against Fibrosarcoma Cells. Gokhru Destroys Melanoma Cell Lines in human beings.

Phyto pharmaceutical is the prevailing field. It is found that with increase in demand of the herbal medicine, they are commercially grown with help of the synthetic fertilizers and pesticides causes poisoning and hence its medicinal value decreases, so growing of the medicinal plants by following vedic method (Biovedic Agriculture) is must to be followed; which deals with using the knowledge of vedas for growing the plant ie Graha Naxatra, Time, Agnihotra Tantra Mantra, Yantra also influence the growth of plant which has been found scientifically in short use of cosmic relation for growth of plant will give the good variety of plant and will have good medicinal value Similarly use of the plants to cure the disease of the medicinal plant Vrikshayurveda is use of the plants (neem, tulsi, aloe, vasa, weeds) for healthy growth of the medicinal plant e.g. Aloe extract is used for fungus existing in cummin seed plant, similarly several other plants are used against the disease of the plants, not only microorganisms effect the health of plant but also the unrequired metal pollutants toxify plant hence the medicinal value also effected so for that Phytoremediation of the soil is done.

SCIENTIFIC APPROACH

We have selected the plant Peristrophe bicalyculatta (RetzNees) which was dully authenticated^[1,2,3] and then Extraction and identification of the active constituents which are plant metabolites, according to their composition, (alkaloids, glycosides, cortocosides, Essential oils Terpenoids, flavanoids, anthocyanins, steroids, vitamins, aminoacids, sugars, saponin etc.) and their effect over the microbes was done.

Plant description as in Adarsh Nighantu written by BAPALAL VAIDYA^[6]

General features:

>It is herbaceous, 3-4 feet in height, stems and branches usually 6 angled more or less hairy, rough in angles, leaves 2-3 inch by 1-1 $\frac{1}{2}$ inch.

>Ovate, acuminate densly linoelate, less hairy above, base usually round, flowers in trichotomus cymes and large laks divaricate, bract beneath calyx-2, oppositely often very unequal. Flowers purple or rose coloured.

≻It is useful in treatment of Jaundice, Typhoid, In Curing Deafness, it has Wound Healing Properties, Sedative, Tuberclosis.

≻It is also useful as an ANTIDOTE^[7,8,9] against Snake Bite

Preliminary investigation^[10]:

It was dully authenticated shade dried, pulverised,





to 60-80 mesh value solvent extracted with increasing polarity and were tested for the active constituents and ash analysis was carried out.

Chemical extractive:

The weight amount of the powdered material was successively extracted in a Soxhlet extractor with increase in polarity. This residue was weighed and per[6]

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Extract	Yield%	Hydrocar bon	Sterol	Fattyacids	Phenols	Sugars	Aminoacids
Pet.ether (60-80)	5.55	+	+	+	_	-	-
Benzene	1.50	+	+	+	-	-	-
Chloroform	2.00	+	+	+	-	-	-
Ether	1.05	+	+	+	+	-	-
Acetone	0.25	-	-	+	+	+	-
Methanol	3.75	-	-	-	+	+	+
Alcohol	6.50	-	-	-	+	+	+
Water	20.25	-	-	+	+	+	+

Ash analysis^[10,11]:-

20 gm of powered material was taken in silica crucible. This was incinerated in a muffle furnace at a temperature not exceeding 450*c until all carbon was removed. The crucible was cooled and ash obtained was weighed to determine its percentage value. The total nitrogen content of the stem was determined by kjeldahl's digestion and distillation method and protein percentage was calculated. The stem was found to contain 8.5% of nitrogen equivalent to 53.69% of total protein.

Sr.No.	Content	%
1	Loss in wt. (110°C)	2.59
2	Ash value	6.50
3	Acid insoluble ash	0.75
4	Iron as Fe ₂ O ₃	0.68
5	Ca as CaO	2.15
6	Magnesium as MgO	2.05
7	Sodium as Na ⁺ flame photometry	0.98
8	Potassium as K ⁺ F.P	1.50
9	Cl	0.54
10	SO_4^{-2}	0.15

ISOLATION OF PHENOLIC CONSTITUENTS^[12]

The natural phenolic constituents includes Phenols, phenolic acid, phenoylpropanoids, Flavanoids, Anthocyanins, Flavanols, Flavenerets, Xanthones, stilbenes and Quionone Pigments, free radicle produce more than 100 disorders but phenolic constituents act as natural antioxidant and helps in its neutralisation.e.g.Pure Cocoa Powder has cancer preventive Phenolic constituents

The presence of the phenolic constituents were detected by

Neutral FeCl_3 giving red, violet, bluegreen coloration

0.1% FeSO_4 + 0.5% Na,K Tartarate ew drops giving blue, green, red to violet coloration

Vanilin H₂SO₄

The total phenolic constituents were determined by Folin Ciocataus reagent method and expressed in terms of GALIC ACID Equivalent

Isolation of phenolic constituent

500 gm of dried ground stem of P.bicalyculata
Extracted in 2 liters 80% boiling methanol for 12 hours
(filtered through cotton)
Reduced to water phase under rotary evaporator
Add HCl (0.05 M), boil in water bath for 15 minutes
Filter through celite with slight suction
-

Dry ethyl acetate with anhydrous Na₂SO₄, filter, wash Na₂SO₄ With 10 ml ethylacetate

Evoporate combined ethylacetate under rotary evporator.

Isolation of phenolic compounds

Several methods for isolation of phenolic constituents^[12,13] from plants have been reported by many workers. In present study, phenolic constituents were isolated using 80% methanol in Soxhlet extractor for 12 hours., the concentrate Methanolic extract was acidified and phenols were isolated using ethyl acetate as solvent^[14] and further characterized by TLC using solvent Ethylacetate-benzene (9:11); HAc-Chloroform (1:9);^[15] these were identified with the standard compounds provided by Dr. M.A.RAGAN (Atlantic RegionalLaboratory, Halifaax USA)

RESULTS AND DISCUSSION

Result of preparative TLC and HPLC reveals the presence of 3-bromo,4-hydroxybenzoic acid and 3-



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bromo, 4-hydroxybenzaldehyde as major brominates phenolic constituents in P.bicalyculata which were further isolated and identified by spectroscopy.

Anti Microbial Activity^[16]

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Phenolic constituents are widely distributed in nature particularly in the plant kingdom. They are generally biologically active and the anti microbial activity is the most common biological property observed from the phenolic substances^{[13].}

It has been reported to be effective on wound healing^[6], this phenomenon can be attributed to presence of the phenolic substances having halogenated compounds.

The phenolic fraction of P.Bicalyculata displayed a significant antibacterial activity against Gram –ve bacterial E.coli, and moderate activity was observed in case of Gram + ve bacteria. In a case of fungal strains, significant activity was observed. The phenolic constituent taken displayed anti microbial activity against gram –ve bacteria E. Coli.(22mm), and a moderate activity was observed in case of gram + bacteria B.Subtillis (15mm) and S.Pyogens (13mm) in case of fungus significant activity was observed against strain A.Niger (20mm).

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