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Antimicrobial activity of Some Ethno-medicinal Plants used by Baiga Tribes from Amarkantak, India

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Abstract

Antimicrobial activity of 05 ethnomedicinal plants extracts were evaluated against six bacterial strains *Staphylococcus aureus* , *Niesseria gonorrohae* , *Pseudomonas aeruginosa*, *Escherichia coli Streptococcus pyogenes Bacillus subtilis.* The collected ethnomedicinal plant were used in folk medicine in the treatment of diuretic stomachache , urinary tract, piles ,high fever , leprosy , ulcers, sexual diseases like gonorrhea and spermatorrhoea, dogbite ,snakebite, skin diseases, respiratory problems asthma , bronchitis, nervous diseases and blood purification. Plants were collected from mekal hills, sonmura ,mai ki bagi, sammbhudhara ,laxmandhara , panchdhara ,dense vegetative forest ,dense rain isolated eco-system, remote valleys and islands and the ethnomedicinal data were gathered from traditional healers who inhabit the study area. The traditional, chemosystematics and methanol extract were obtained by maceration method with antimicrobial activity was found using disc diffusion method phytochemical anaylysis, Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). All microorganism were obtained from (ATCC), American type culture collection.The result indicate that out of 05 plants exhibited antimicrobial activity against one or more of the tested microorganism we observed that extract concentrations spanning from 25 µg/ml to 2 mg/ml or even 40 mg/ml. Concentration of alkaloids, carbohydrates, flavonoids, saponins, terpens steroids associated to antioxidant activities and thus have curative properties. MIC and MBC for all 5 crude extracts (72%) showed positive results against bacterial strains.This study evaluate the antimicrobial ,phytochemical and Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) activity of the some ethnomedicinal plants used in folkoric medicine compared to all test showed significant activity against tested microorganism . This study also showed that *Selaginella bryopteris* (Amarbatoooti Sanjivini), *Lygodium flexuousm* (Kalijar) *Adiantum philippense* (Kalijhant), *Drypteris eochleata* (Jatashankari), *Tectacria coadunate* (Jatamasi) could be potential sources of new antimicrobial agents.

Keywords: key words, ethnomedicinal plant, Antimicrobial, Phytochemical,diseases

1. Introduction

More than 80% of the world's population uses natural medicines and depends on medicinal plants for health care. In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded within and across the countries. At present, 90% collection of herbal raw drugs used in the manufacture of Ayurveda, Siddha, Unani, and Homeopathy systems of medicine is largely from the wild out of which 70% collection involves destructive harvesting. Due to this spurt, medicinal plants are being overexploited and many of them are pushed to the brink of extinction. Many medicinal plants are highly sensitive to the level of harvest and fragility of the ecosystem (Pandey A K et al. 2010).

Amarkantak is one of the world's focuses for conservation located at 22.67°N 81.75°E. It has an average elevation of 1048 metres (3438 ft) from sea level this biomass should be studied in terms of

pharmacological or biological activity. Approximately 40% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. The systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. The temperate climate and the equitable distribution of rain make Amarkantak an ideal plateau for dense vegetation cover 21 pteridophytes 7 of these species were new for Central India and 14 for Madhya Pradesh (Khare P B 1989 and Saxena O P 1970).

In isolated eco-system, such as remote valleys and islands, there is an extremely wide variety of floristic genera, but with the proportion of species to genera being very small. This is because each genus is unique to itself and does not proliferate and subdivide into many species. The eco-system of Amarkantak is truly unique, closely resembling that of isolated valleys or islands, because whereas the proportion of species genera is 13:1 in the world, in India it is 7:1 and in Amarkantak it is 15:1. This makes every genus in Amarkantak of great medicinal importance because if a particular plant becomes extinct the genus itself will die because it does not live through any of its variant species are now gravely endangered, especially because it is highly localised around Amarkantak, and it requires total protection. The region is tribal dominant and tribes are Gonds, Bharia, Bhils and Baiga's they are dependent on forest products for their survival they used their traditional knowledge and indigenous systems of medicine for the treatment of various diseases (Sweta Singh et al. 2005, Dixit R D 1947). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Kloucek P et al. 2005). The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Sandhya B et al. 2006). Rural communities in particular baiga tribes of Amarkantak depends on plant resources,claim that their medicine is cheaper and more effective than modern medicine.

The reports on the use of plants in traditional healing through tribes interview, discussion, personal contacts and keen observations i.e. *Selaginella bryopteris*, (Amarbatoooti Sanjivini;used for gonorrhea and venereal diseases) *Lygodium flexuousm*,(Kalijar; leaves applied for piles, rheumatism, sprains, ulcers, cut wounds and scabies also cure gonorrheao ,spermetaorrhoea and menorrhagia ,spores cure high fever.) *Adiantum philippense* (Kalijhant; uses for leprosy, dysentery, ulcers, asthma, bronchitis as well as dogbite and snakebite), *Drypteris eochleata*, (Jatashankari ; rhizomes shows antifungal activity and used as antidote and also applied for cuts, wounds ,ulcers, blood purification) *Tectacria coadunate* (Jatamasi; used in -respiratory disorders).

The principal aim of the present study was to screen extracts obtained from the Amarkantak forest for antibacterial activity against the phytochemical research based on ethnopharmacological informations is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Kloucek P et al. 2005). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul OT 2002) screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. This paper reports the results of a survey that was done based on folk uses by traditional practitioners in Amarkantak Mekal Plataue along with bioassay test for antimicrobial activity.

2. Material and Methods

2.1 Plant collection and extract preparation

All the plant were collected with the Amarkantak mekal plataue and Achanakmar biosphere region of Chhattisgarh five plants were used for organic and aqueous extracts were obtained from plants native to the tribal region. Plants were collected using a traditional[5] and chemosystematic[6] approach. Plant parts were collected according to the biomass availability. Plant material was dried and ground before being submitted

to 24-h maceration with methanol: dichloromethane (1:1) followed by 24-h maceration with water, resulting in four extracts from each plant material *viz, Selaginella bryopteris* (Amarbatoooti Sanjivini), *Lygodium flexuousm* (Kalijar) *Adiantum philippense* (Kalijhant), *Drypteris eochleata* (Jatashankari), *Tectacria coadunate* (Jatamasi).

2.2 Phytochemical test

Mayer test for Alkaloids take 0.135g of mercury bichloride, and 0.05g potassium iodide, are dissolved in 100 mL of water; this is used as a test for alkaloid, with which it gives a white precipitate. $FeCl_3$ test for Tannin take $FeCl_3$ solution and add 2 ml of extract. It gives deep blue color. This indicates the presence of tannin. Frothing test for Saponin take 300mg of extract and boil with 5ml water for 2 minutes. Mixture was cooled and mixed vigorously and left for 3 minutes. The formation of froth indicates the presence of saponin. Salkowski test for Steroids take 2ml of plant extract and add 2ml of chloroform and 2ml of conc. sulfuric acid. Shake it well. Chloroform layer appears red and acid layer shows yellow fluorescence. Sodium hydroxide test for flavonoids addition of increasing amount of sodium hydroxide to the residue shows yellow coloration which decolorizes after the addition of acid (Turnidge JD et al. 2003). Benedicts's test for Carbohydrates add 1 ml of the solution to be tested to 5 ml of Benedict's solution, and shake each tube. Place the tube in a boiling water bath and heat for 3 minutes. Remove the tubes from the heat and allow them to cool. Formation of a green, red, or yellow precipitate is a positive test for reducing sugars (Skene et al. 2006, Andrews, J M 2001 and Policegoudr RS et al. 2007).

2.3 Antimicrobial assay

Broth microdilution method was used to screen the 5 plant extracts. The inoculums was prepared at the concentration of 10^{-2} CFU/ml, starting from a 0.5 McFarland (or 108 CFU/ml) prepared from fresh colonies of bacteria as described below[7]. *Staphylococcus aureus, Niesseria gonorrohae, Pseudomonas aeruginosa, Escherichia coli Streptococcus pyogenes Bacillus subtilis* were the bacterial strains tested (HiMedia Laboratories Pvt. Limited Mumbai). The bacterial inoculum of each was obtained from fresh colonies grown on Mueller Hinton agar plates. Each strain was inoculated into 5 ml of Muellen Hinton broth in order to obtain a concentration of 1.5 x 108 CFU/ml. The inoculums were then diluted to 1.5 x 102 CFU/ml. One hundred and ninety microliters of this suspension was transferred to each microplate well. Ten microliters of each extract solution was added to the microplate wells and incubated at 35ºC for 18 to 20 h. Extracts were prepared to 20 times the desired test concentration (2 mg/ml) in water or 50% DMSO solution. The extracts were screened at a concentration of 100 µg/ml. Extracts that showed inhibitory activity at this concentration were submitted to a subculture of the broth media in Mueller Hinton agar (HiMedia Laboratories Pvt. Limited Mumbai) in order to evaluate bacterial growth (Macfoy C A et al. 1990, Maikere-Faniyo R et al. 1989, Biavatti MW et al. 2001, Alves TM et al . 2000, Gnan SO et al. 1999 and Nakamura CV et al . 1999).

2.4 Determination of minimal inhibitory concentration and minimal bactericidal concentration

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined for the extracts that showed total growth inhibition using the protocol described above. Extract concentrations of 10 to 100 µg/ml in steps of 10 µg were evaluated. The concentration at which there was no visually detectable bacterial growth was taken as the MIC, and the concentration at which there was no bacterial growth after inoculation in Mueller-Hinton agar was taken as the MBC (Eloff JN 1998).

3. Results

The result of phytochemical screening is presented in (Table 1.) this reveals moderate concentration of alkaloids, carbohydrates, flavonoids, saponins, terpens and steroids some of which chemical compounds have been associated to antioxidant activities and thus have curative properties. Bacterial growth inhibition was confirmed after inoculation in Mueller-Hinton agar. Many extracts showed some degree of inhibition of bacterial growth at concentrations of 100 µg/ml, represented by "++". The MIC and MBC of these extracts are currently being obtained. (Table 2) lists the five plant extracts that showed antibacterial activity against *Niesseria gonorrohae* ATCC 43069 , *Staphylococcus aureus* ATCC BAA 1026 , *Pseudomonas*

aeruginosa ATCC 9027 (HiMedia Laboratories Pvt. Limited Mumbai)and their respective MIC and MBC. (Eloff JN 1998 and Suffredini IB et al. 2002)

4. Discussion

Plant extracts have been studied against bacteria for years, but in a more intensified way in the last three decades. During this period, a lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African, and Asian plant drugs (9-15). During the late 1990's, a large number of manuscripts describing methodologies of screening took part and resulted in more than 30 articles representing antibacterial extracts obtained from Asian and African native plants, but only a few studies relating antibacterial activity of Amarkantak plant extracts were found. Amarkantak is home to more than 20% of the world's biodiversity and only a few species have been submitted to any sort of large-scale biological screening. We collected a substantial amount of information about the antimicrobial activity of 5 Amarkantak plant extracts which have been collected. In order to establish the extract concentrations to be used during the screening, we observed that extract concentrations spanning from 25 µg/ml (Eloff JN 1998) to 2 mg/ml(Loganga Otshudi A et al. 2000) or even 40 mg/m (Gnan SO et al. 1999) have been used. We screened the crude extracts using a concentration of 100 µg/ml (Suffredini IB et al. 2002). Such concentration is nowadays considered the proper concentration an antimicrobial extract should present (Table 3). The dilution test is a very precise technique that permits us to work with such a low concentration.

Only 3 of 5 extracts showed bactericidal activity: extract *Selaginella bryopteris, Lygodium flexuousm* against *N. gonorrhea Lygodium flexuousm* against *S. aureuas* and *Adiantum philippense ,Drypteris eochleata, Tectacria coadunate against P.aeugoginosa*, Several crude extracts apparently inhibited bacterial growth. Among them, we are currently determining the MIC and MBC for all 5 crude extracts (72%) showed positive results against *S. aureus*, *N. gonorrhea*, *P. aeruginosa*. Extract (MIC and MBC), obtained from the stem of a *Selaginella bryopteris, Lygodium flexuousm* plant, showed antibacterial activity against *N. gonorrhea* (MIC = 160 and 140 µg/ml respectively; MBC = 180 and 170 µg/ml). Extract *Adiantum philippense ,Drypteris eochleata, Tectacria coadunate* (MIC = 90 ,80,60 µg/ml; MBC = 140 , 80 ,70 µg/ml respectively), obtained from the stem of a plant, showed activity against P. *auroginosa*. The three active extracts are going to be bioguide fractionated in order to identify their active substances as well as the remaining extracts, whose MICs were ≤500 µg/ml (Kim HK et al.1970, De Tommasi N et al.1993 and Hamilton-Miller JM et al. 1995). All the species have been studied phytochemically and biologically, and beta-sitosterol, betulinic acid and sericic acid have been isolated from their stem bark extracts. (Hess SC et al. 1999). These substances have shown antibacterial activity against *P.auroginosa* and *S. aureus*. It is a matter of major national interest to study the potential of Amarkantak forests in offering new lead antibacterial compounds that can be further synthesized and have their activity improved. Thus, we strongly hope to contribute to the conservation and protection of the biodiversity of our forests and to the development of the Amarkantak community as a whole. Further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these species. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Several plants used by Baiga tribe exhibit some degree of antibacterial activity towards gram-positive bacteria such as, *Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis*. These plants could serve as useful sources for new antimicrobial agents.

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Table No.1. Amarkantak flora are Ethnomedicinaly used by local tribes.

S.No.	Botanical Name/Family	Natural habitat	collection site	Plants parts used	Ethnomedicinal uses
1.	*Selaginella bryopteris* (L.)Baker (Selaginellacea) Local Name: Sanjivini	Heavy rocks boulders , deep forest Amarkantak	Forest of Amarkantak Panchdhara	Leaves dried plant with Tobacco	Paste of leaves used in gonorrhea, spermatorrhoea, leucorrhoea, diuretic, stomachache, urinary tract inflammation in children.
2.	*Lygodium flexuousm* (L) Sw. (Lygodiaceae) Local Name :Kalijar	Grow on Bushes and trees, Sandy Soils	Forest of Amarkantak Mai ki Bagia	Stem, Rhizome leaves, spores	Antibacterial property. Boiled fronds used for Carbuncles, rheumatism ,sprains, scabies ulcers and cuts wounds. Extract of stem and rhizome is taken for curing sexual disease like gonorrhoea and spermatorrhoea. Fresh leaves extract for piles. Spores cure high fever.
3.	*Adiantum philippense* (L) Adiantaceae Local Name:Kalijhant	Under moist condition , Low mountainous region	Forest of Amarkantak near Sambhudhara	Rhizomes, leaves	Fronds extract for fever , asthma , bronchitis ,dysentery ,epileptic fits ,leprosy ulcers and erysipelas. Powder of rhizome as an antitode against dogbite and snakebite. Leaves extract applied for stomach for clear and early release of urine. Died rhizome for women during menstrual period sterility.
4.	*Drypteris eochleata* D.C. (Ham.ex.D .Don) C. chr. Dryopteridaceae Local Name:Jatashankari	Stream sides,Amanala, laterite soils	Amanala Laxmandhara Panchdhara	Rhizome, Leaves stems stipe	Rhizome as Antifungal property ,used as antitode. Extarct of dried rhizome is given in epilepsy and leprosy . Paste of fresh rhizome ,stem stipe is applied for cuts, wounds, ulcers, swelling and pains. Fresh Paste and rhizome and fronds used in snakebite and dogbite. The decoction of dried rhizome tem stipe used for blood purification.
5.	*Tectacria coadunate* (Wall.ex.Hook.et grev.) C.chr (Tectariaceae) Local Name: Jatamasi	Rocks Crevices boulders in moist places along the stream	Sonmura Mai ki Bagia	Rhizome Leaves	Anthelmintic, fresh rhizome and fronds paste is used in insect bites or getting relief in centipede bite. Fresh rhizome and stipe in stomach pain, Gastrointestinal disorders of worm in children .Extraxction of dried rhizome ,stem and stipe is used in respiratory disorders like cold,cough ,asthma and bronchitis.

Table No. 2. Phytochemical screening of the given plant

S.No.	Contituents	Leaves Extracts	Rhizome	stem	Roots
1.	Alkaloids Meyer's, test	++	+	++	++
2.	Carbohydrates, Benedict's test	++	++	+	++
3.	Flavonids ,Sodium hydroxide test	++	+	+	++
4.	Saponins , Frothing test	++	++	+	+
5.	Steroids ,Salkowski test	++	++	++	+
6.	Tannins ,$FeCl_3$ test	+	++	++	+

Key (-)= Negative (absent),(+) = Positive (Slightly present) (++) = Positive (moderately present)

Table No. 3. Antimicrobial activity of plants extract from the Amarkantak forest that showed strong activity, and their corresponding minimal inhibitory and minimal bactericidal concentrations.

Plants Samples	*Selaginella bryopteris* (Amarbatoooti Sanjivini)	*Lygodium flexuousm* (Kalijar)	*Adiantum philippense* (Kalijhant)	*Drypteris eochleata* (Jatashankari)	*Tectacria coadunate* (Jatamasi)
Number of extracts evaluated	**4**	**4**	**4**	**4**	**4**
Extract classified as ++++ against *Niesseria gonorrohae* ATCC 43069	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**
Extract classified as ++++ against *Staphylococcus aureus* ATCC BAA 1026	**-ve**	**+ve**	**-ve**	**-ve**	**-ve**
Extract classified as ++++ against *Pseudomonas aeruginosa* ATCC 9027	**-ve**	**-ve**	**+ve**	**+ve**	**+ve**
Minimal inhibitory concentration (µg/ml)	**160**	**140**	**90**	**80**	**60**
Minimal Bactericidal concentration (µg/ml)	**180**	**170**	**140**	**80**	**70**

Activity was measured by the micro dilution broth assay ++++ indicate that the extracts caused total growth inhibition