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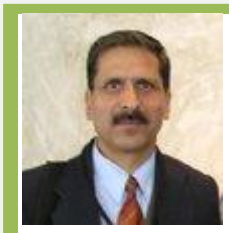

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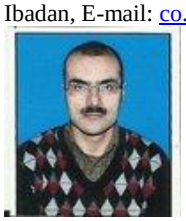

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Research Article

IDENTIFICATION OF CHEMICAL MARKERS FROM LICHEN SPECIES FOR MEDICINAL USES

Muhammad Arif*[1], Veenita Tomar[2], Manjoosha Srivastava[3], D.K. Upreti[4] and Sangeeta Srivastava[5]

*[1,2,3] Phytochemistry Division, [4] Lichenology Laboratory,
CSIR-National Botanical Research Institute, Lucknow, U.P.
[5]Department of Chemistry, University of Lucknow, Lucknow, U.P.
Corrosponding author: arif28dawn@gmail.com

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Abstract

Lichens are symbiotic association of an algae and fungus. They are stable, self supporting and slowest growing pants. They are mainly distributed in rainforest, desert, mountain, snowy areas, and sea shores. They have many biologically active chemical markers like depsides and dibenzofuran. Studies reveal that extract yield varied from 5.92% -14.44% in acetone and 4.90%- 13.20% in 50% ethanaol. The secondary marker isolated from these species is usnic acid (1.94%), fumarprotocetraric acid (1.88%) respectively. The study leads to metabolite profiling of lichen species, establish standard protocols of extraction & determine to select specific major bioactive markers for isolation, large scale production & utilization for food and pharma industries for production of herbal medicines.

Keywords: Chemical marker, fumarprotocrtraric acid, herbal medicicine, usnic acid.

Introduction

The frequent and long term use of synthetic medicine has numerous complications some time causes resistance for health so we need to find new medicine from herbal origin. There are some drug of synthetic origin has already replaced successfully but this is not so sufficient. Unlike synthetic medicine herbal medicine are also beneficial for whole organism so there are need of more herbal drug as we know they has no side effect like synthetic drugs. In search of herbal medicine lichen species can helpful like higher plant groups in many ways. Lichens are symbiotic association of an algae and fungus. They are stable, self supporting and slowest growing plants (P. Sivastava, 2013). They are mainly distributed in rainforest, desert, mountain, snowy areas, and sea shores. A number of 25,000 lichen species have been used as food, spices, dyes, floral decoration, pollution monitoring (Ingolfsdottir, 2002; Mitrovic, 2011) in production of alcohol, perfume industry and drugs of popular medicine (Vartika 1973; Richardson 1988). Lichens have a vast range of chemical

markers which is nearly 1350 of chemically diversified groups such as depsones, depsidone,depsides and dibenzofurans (Asahina and shibata, 1954; Culberson, 1969; Huneck and Yoshimura 1996) beside these groups they also have alkaloids, phenolis, flavonoids, saponin, terpenes, carbohydrates, proteins, and amino acids. They possess various biological activities viz. analgesic, antipyretic, antitumor (Choudhary, 2005) antiviral, antibiotic, anti-allergic, anti-herbal, they inhibit growth of plants; as well as numerous enzymes (Lawrey, 1986; Halama, 2004; Oksanen, 2006; Rankovic, 2008) and also act as curatives for many other diseases. In this context, lichens viz. *Usnea longissima* and *Cladonia furcata* were studied for bioprospecting their phytochemicals. Usnic acid and fumarprotocetraric acid are the major chemical markers isolated from *Usnea longissima* and *Cladonia furcata* respectively. Usnic acid is a very unique compound used in many preparations like toothpaste, deodorants, mouthwash, sunscream, parfumary and cosmetics it also has many biological activities such as antiviral, antimicrobial, antiprotozoal, antiproliferative, anti-inflamatory and analgesic (Ingolfsdottir, 2002). Fumarprotocetraric acid also has some biological activities like antioxidant, antimicrobial and anticancer (Rankovic, 2011).

Materials and Methods

Collection

The lichen sample *Usnea longissima* was collected in November 2012 and recorded the accession no. 28382, specimen LWG no. 12-018850 and *Cladonia furcata* was collected in September 2012 and recorded the accession no. 28559, specimen LWG no. 12-018750 both samples were collected by lichenology laboratory of CSIR- NBRI from Uttarkashi district of Uttarakhand, India.

Extraction

Lichen thallus 0.5g was extracted in 100 ml of acetone and 50% ethanol respectively for 24 hrs. Then extracts were filtered through a whatman filter paper no 42 (125mm) and concentrated on rotavapour (Buchi, R 200 V800, USA) under reduce pressure of 980 rpm at 40^0c temperature. Further extracts were freeze-dried in lyophilizer (Labconco, USA) to obtain completely dry solid extract.

Qualitative estimation of chemical markers

Chemical markers present in different solvent extracts were identified through TLC and isolated through column chromatography.

Solvent systems used in TLC

(A). Toluene: 1,4-Dioxane: Acetic acid (18:4.5:0.5)
(B). Hexane: Di-ethyl ether: Formic acid (13:8:2)
(C). Toluene: Acetic acid (17:3)

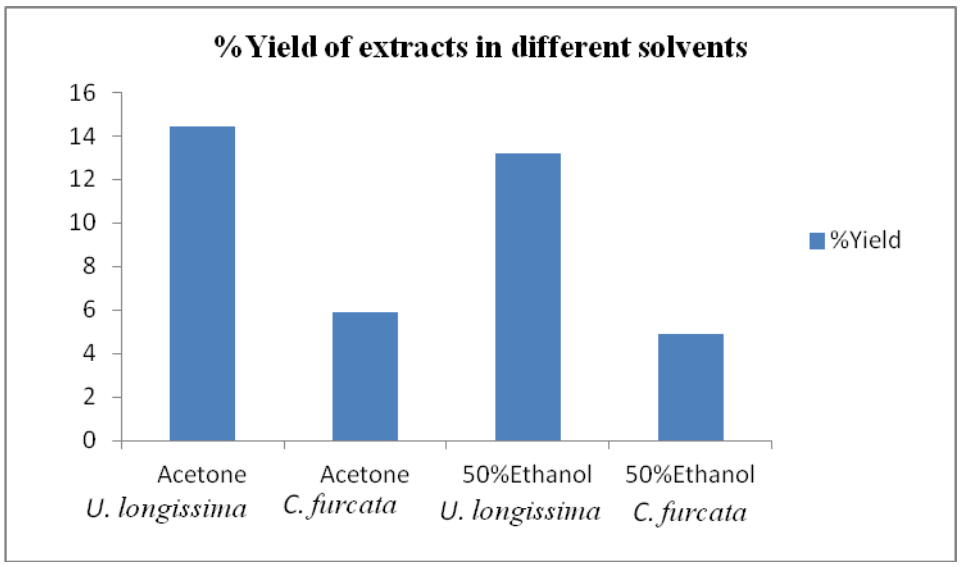
Isolation of chemical marker

Column was packed with silica gel (60-120 mesh) using hexane and 0.05g extract was loaded for each species. The extracts were run in hexane-chloroform (9:1) and chloroform-methanol (9:1-1:9) as eluting solvent. Total 60 fractions were fractionated from these two species and checked in TLC and Rf value of markers were calculated. Many minor chemical marker were separated and identified in different fractions but the major chemical marker viz. usnic acid and fumarprotocetraric acid were identified in

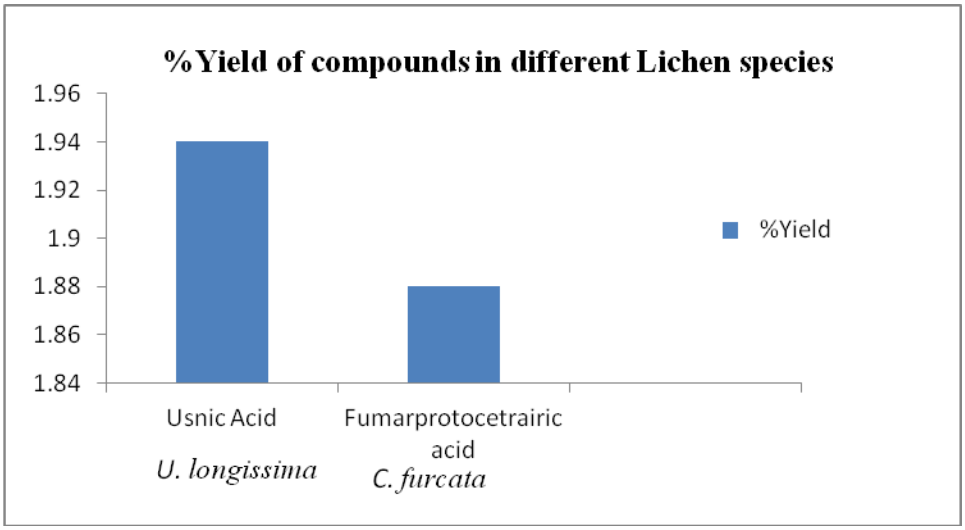
chloroform-methanol fraction (8:2 and 7:3) respectively.

Result and Discussion

The parcentage yields of extracts were calculated for the identification of usnic acid, fumarprotocetraric acid and other chemical markers. Studies revealed that extract yield varied from 5.92% -14.44% in acetone and 4.90%-13.20% in 50% ethanol, shown in Graph 1. The chemical markers isolated from these two species were usnic acid and fumarprotocetraric acid and their yield was found 1.94% - 1.88% respectively and shown in Graph 2.



Graph-1. Percentage yield of different solvent extracts in Lichen species



Graph-2. Percentage yield of isolated compounds in different Lichen species

Acetone and 50% ethanol was found good for the extraction and isolation of compounds. The chloroform-methanol fraction i.e (8:2 and 7:3) was found better eluting solvent for isolation of usnic acid and fumarprotocetraric acid which is shown in fig-1 and fig-2.

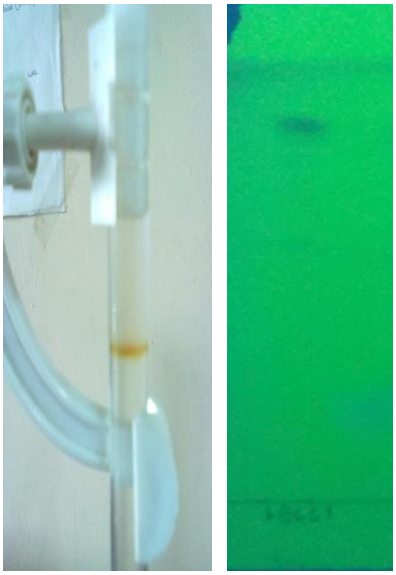


Fig-1. *Usnea longissima*

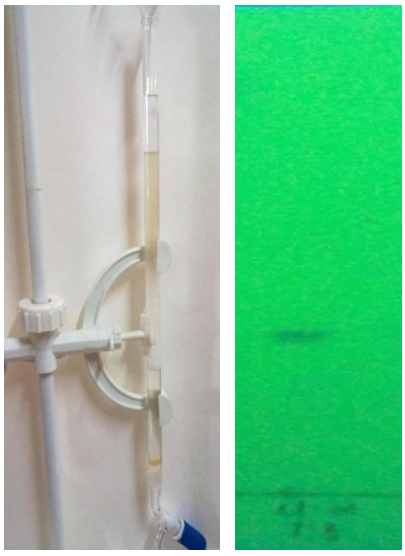


Fig-2. *Cladonia furcata*

Fig.1 and 2. Isolated pure compound through column chromatography

Since the study is emphasis on separation of chemical markers and identification through thin layer chromatography of lichen species as we discussed before that the uses of lichen species and their chemical marker and we know these chemical markers of lichen species are very useful for mankind as they have numerous biological activities and ability to cure many diseases from centuries till date. Thus lichens and its chemical markers are very promising for the purpose of pharmaceutical industries in making herbal drugs, food products and their potential use.

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Research Article

AN INTRODUCTION OF NEW WEEDS AT UTTARKASHI DISTRICT OF UTTARAKHAND STATE

Mahendra Pal Singh Parmar, Prerna Pokhriyal, Naresh Singh Chauhan and Richa Badhani

Department of Botany, Govt. P.G College, Uttarkashi, U.K.
*Corresponding author: mahen2004@rediffmail.com

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Abstract

Weed commonly called *khar-kabad* in Uttarakhand or *kharpatawar* in India and worldwide. Any unwanted plant which reduces the productivity of our commercial crop, farmers is often concerned that weeds may reduce crop yields. Weeds use the same nutrients that crop plants use, often in very similar proportions. They also use resources such as water, sunshine and space that might have gone to crops. The more similar the weed and crop requirements, the more they will compete for those resources. Weeds that compete aggressively with crops reduce their yield. Weeds damage the crop yield and they are highly unwanted. Four factors are especially important: density, timing, size and chemistry. For instance, at very high densities, green foxtail plants tend to compete strongly with each other and thus remain very small. These small plants probably have little competitive effect on the crop even when there are many of them. At medium densities, green foxtail plants grow larger and can severely reduce crop yields. In this example, a reduction in weed numbers may actually increase the weed problem.

Keywords: Weeds, nomadic grazing, introduction of new weeds, fertilizers, fungicides

Introduction

Weed biology relates to the plant attributes such as morphology, seed dormancy and germination, physiology of growth, competitive ability and reproductive biology. Knowledge of weed biology is essential for development of both economically and environmentally acceptable weed management systems. It is also essential to understand and predict how weed species, populations and biotypes evolve in response to the selection pressure primarily due to agricultural and related practices. Weed identification is the first step in understanding their biology.

Timing of weed-crop competition is important. Ecologists have defined a critical period of weed competition. This is the time when the weed reduces crop yield. Weeds that are removed before the critical period, or that emerge after the critical period do not cause any appreciable yield loss. The exact timing of this period is not an "inherent property of the crop" and varies for different crops, for different weed species, and under different conditions such as year or location. In general, weeds should be removed at early crop growth stages. Early weed removal was found necessary to protect field yield. Relative timing of crop and weed emergence is very important in determining

the magnitude of yield loss from weed competition (Singh et.al.) When it comes to plant competition, generally the first one out of the ground wins. Competition from wild oat resulted in a 17% yield loss in barley when it emerged five days before the crop compared to a 3% yield loss when wild oat emerged five days after crop emergence. Weed size is partly a matter of timing. Weeds that emerge before the crop are generally larger and better established than those that emerge after the crop. This gives them greater access to soil and spatial resources, and thus they do more damage to crop yield. Size also varies among species. For instance, Canada thistle plants are naturally much larger, and likely to cause more yield loss, than thyme-leaved spurge plants. Size also depends on plant nutrition, disease, and pests. Some weeds may limit crop development through chemical means, or allelopathy, either while they are alive, or as they decompose. Some weeds, for example (Naidu, 2012) or quack grass, release chemicals that inhibit their neighbors . This affects their competitive relationships. Weeds can cause problems other than crop yield loss. Some weeds are poisonous and can taint food and feed crops. For example, wild mustard seed cannot readily be removed from canola, and can flavor the resulting canola oil if crushed with the crop seed. Stinkweed in feed for dairy cattle produces off-flavors in milk.

Weeds that remain green at harvest, especially those with fibrous stems, can interfere with harvest. The problem varies with both the crop and the weed. A low-growing weed like wild tomato causes very little problem in a cereal crop because most of the plants are below swath height. In a crop like lentil, chickpea, or bean, severe harvest difficulties may occur. The low cut means that wild tomatoes are harvested with the crop, and they can stain the pulse and clog the machinery. Weeds like wild buckwheat that twine through a crop can also be problematic. Weeds can harbour problem insects and crop diseases. For instance, mustard-family weeds can carry over canola diseases, making rotation a less effective tool for disease management. Immature weeds can interfere with harvesting operations. Weed seeds in harvested crops cause dockage and increase risk of spoilage. This can reduce crop value, or increase shipping costs. Weeds in grasslands are generally those that are less palatable. They increase with grazing, because the livestock graze them less than the more palatable plants. Over time, this reduces range productivity for livestock. Weeds such as smooth brome or purple loosestrife can compete aggressively with native vegetation, and replace it.

Materials and Methods

Uttarkashi district is located in the catchment of two major river system of India i.e. Ganga,Yamuna and tributaries. The district lies between N 30^0 27’ latitude and E 78^0 54’ to 79^0 25’ longitude and has a total geographical area approximately 8016 sq. km. Among of which 21% land are used fr Agriculture or Horticulture. So traditional crop like cereal plants are *Tritium vulgare* (wheat), *Oryza sativa* (rice) and that make about 75% of total cereals of Uttarkashi rest *Zea maiz* (Maize). The minor cereal plants viz *Elusine corsicana* (samak) and pseudo cereals like *Fagopyrum esculentum* (kutu), *Amaranthus causation* (Ramdana) etc are included in remaining 25% of cereals. More than eight species of family Papilionaceae viz. *Dolichos lab lab* (Sem), *Glycine max* (soybean), *Pisum sativum* (matar), *Lens esculentum* (masoor), *Phaseolus munga* (urd), *Phaseolus radiatus* (moong), *Vigna*

sinensis (Rajama) etc were produced by organic methods except Rice and Wheat.

After heavy infestation of chemical, fertilizer, insecticides, fungicides and seeds from outer agencies, it has been observed by us weeds crops drastically increased however production of the crops increases in the area through the support of hybrid seed, chemical, fertilizer, insecticides, fungicides etc.

Result and Discussion

During the survey at Uttarkashi villages i.e Gainwla (Barshali), Chinyali, Matli , Dhrashu etc following weeds were observed either fields and nearby area of farming fields during Ravi, Khariff and Jayad session.

S.N	Botanical name	Local Name	Life Span	Habit	Family
1	*Amaranthus viridis*	Junga-li chaulai	Jan-Dec	Erect	Amaranthaceae
2	*Avena fatua L.*	Jawatu	Apr-May	Erect	poaceae
3	*Chenopodium album*	Bath-ua	Jan-Dec	Erect	Euphorbiaceae
4	*Coronopus didymus L. Smith*	Jungle ajwan	Mar-Oct	Erect	Brassicaceae
5	*Convolvulus arvensis*	Heyranpatu	Sep-April	Climber	Convolvulaceae
6	*Cleome viscose L.*	Jakhya	Jul-oct	Erect	Cleomaceae
7	*Cynodon dactylon L. pers*	Dubla	Apr-Jul	Grass	Poaceae
8	*Cyperus compressus L.*	Murya	Jul-Nov	Sedge	Cyperaceae
9	*Cyperus rotundus L.*	Motha	Jul –Dec	Grass	Cyperaceae
10	*Eclipta prostata L.Mant.*	Bhangiri	Mar-Sep	Prostrate	Asteraceae
11	*Eleusine indica L. Gaertn*	Jharnpriya-kodu	Jul-Nov	Grass	Poaceae
12	*Euphorbia heterophylla L.*	Dudhya	Feb- Aug	Erect	Euphorbiaceae
13	*Euphorbia hitra L.*	Chota-dudya	Sep- Oct	Erect decumbent	Euphorbiaceae
14	*Lantana camera L.*	Kuri Ghas	Jan- Dec	Erect	Verbenaceae
15	*Malva parviflora*	Soncheli	Jan-June	Prostrate	Malvaceae
16	*Medicago polymorpha*	Ghadu	Aug-Sep	Decumbent	Fabaceae
17	*Melilotus alba Medikus*	Safed senji	Aug-Oct	Erect	Fabaceae
18	*Melilotus indica(L) Allioni*	Ban methi	Aug- Oct	Erect	Fabaceae
19	*Oxalis latifolia Humb.*	Bilmoria	Jan- Oct	Erect	Oxalidaceae

20	*Polygonum plebeium*	Dondya	Jan-Dec	Erect	Polygonaceae
21	*Rumex hastatus*	Almoro	Feb-Oct	Erect	Polygonaceae
22	*Solanum nigrum*	Makoi	Aug-Sep	Annual	Solanaceae
23	*Tridex procumbens*	Kanphuli	Jan-Dec	Erect	Asteraceae
24	*Anagallis arvensis*	Billi booti	Feb-Oct	Erect	Primulaceae
25	*Asphodehus tenuifolius*	Bhokat piazi	Jan-Dec	Erect	Asphodeliace
26	*Achyranthes bidentata*	Chicheree	Jan-Dec	Annual/ Erect	Amaranthaceae
27	*Asparagus racemosus*	Satrawar	April – October	Perennia l	A.racemosus.
28	*Centella asiatica*	Brahmi butti	April - October	Prostate	Asteraceae
29	*Carthamus oxycantha*	Pohli,Kandiari	April - September	Annual weed	Asteraceae
30	*Fumaria indica*	Shahtra, Pitpapra	Jan-Dec	Semi erect, Annual weed	Fumariaceae
31	*Glium aparine*	Wambooti	Jan- Dec	Annual/ erect	Rubiaceae
32	*Lathyrus aphaca*	Dokanni	Annual	Jan - May	Papiolanaceae
33	*Lathyrus sativus*	Kurri, Chraal ,Kasseri	Annual	Dec- May	Papiolanaceae
34	*Lepidium sativum*	Halon	Annual	Dec -Jan	Brassicaceae
35	*Phalarish minor*	Dumbi sittee	Annual	Jan-July	Poaceae
36	*Saponaria vaccaria*	Takla	Annual	July - Dec	Caryophyllacea e
37	*Spergula arvensis*	Van dhaniya Kalri booti	Annual	Dec - Jan	Spergulaceae
38	*Stelleria media*	Stelphullan booti	Annual	July - March	Caryophyllacea e
39	*Eupatorium adenophorum*	Kala bansa/ Bhangu	Annual	Erect	Astraceae
40	*Ageratum houstonianum*	Fulmundya/parde shi ghas / flossflower	Annual	Semi Erect	Asteraceae

During the survey authors observed that the major reason of Weed invading in Uttarkashi is Nomadic Grazing grazed by Gujjar,Khadwal , Bakkarwal and Gaddi etc. After Holi to Deeepawali (March to October) they carried their grazing animal i.e Buffalo, Sheep,Goat, Horses etc at high elevation by roads . These animal carry different kinds of weed seed or genetic material with their tail, horn,hair and skin and inside of the animal (Endozoochory/ Epizoochory).

Conclusion

It has been observed by authors' 20% new weed species invaded and 10% old species replaced by new weed species and most of new species are occurred from outside seeds, nomads (Shepherded/Gujjars). These Shepherded and Gujjars comes here every march of the year from plain area with their sheep, goats, buffalo, horse , cow and bullock etc. carrying with different kinds of weeds through their animals external organs i.e hair, tail, horns, foot etc. Weeds are attached to their external organs and reached till grater Himalaya (above 4000Mtrs). Authors suggested that nomadic grazers should clean their extremities'. It has also been obserbes after using chemical, fungicides, the numbers of weed increases and our traditional crops are almost weed less.

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Research Article

COMPARATIVE STUDY OF ANTHRAQUINONE GLYCOSIDES IN *CASSIA* SPECIES AND THEIR ANTIMICROBIAL ACTIVITY

Veenita Tomar*[1], Muhammad Arif[2] and Manjoosha Srivastava[3]

*[1, 2, 3] Phytochemistry Division, *[1]Academy of Scientific and Innovative Research
CSIR-National Botanical Research Institute, Lucknow, U.P.
*Corresponding author: veenita27tomar@gmail.com

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Abstract

The Genus *Cassia* belong to family leguminoseae (sub- family Casealpinaceae) comprises of about 500 species. Out of these 23 species occur in India. *C. fistula* and *C. javanica* are rich in anthraquinone glycosides. Leaves of these species are traditionally known to combat skin problems i.e. cuts, burns, wounds and are purgative. The present study deals with comparative, qualitative and quantitative estimation of anthraquinone glycosides in different extracts of leaves and antimicrobial activity of their extracts. On the basis of phytochemical screening methanolic extract was found potential for the estimation of anthraquinone glycosides. Methanolic extract of leaves was found to have maximum anthraquinone glycosides in *C. javanica* as compared with *C. fistula*. Aloe-emodin was major identified marker on the basis of Thin Layer Chromatography. Thus anthraquinone glycoside is maximum in methanolic extract of *C. javanica* leaves as compared with *C. fistula* leaves. The potential extract of *C. fistula* leaves having significant activity against bacterial and fungal strains may be utilized for its prospection as antimicrobials for therapeutics.

Keywords: Anthraquinone glycosides, antimicrobial activity, thin layer chromatography, therapeutics

Introduction

In present scenario, elevation of environmental pollution at global level affects the health of living people, majorly in developing countries by infectious diseases. Multi-drug resistant microbial strains and appearance of strains with reduced susceptibility to antibiotics are continuously increasing that is a big threat to health of living community throughout the world (B.R. Pandey 2015). Although large numbers of synthetic antimicrobial agents have been discovered, they have side effects and pathogenic microbes are constantly developing resistance to these agents. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security (Joy el al., 2001). Plants are the highest source for bioactive herbal secondary metabolites which have been exist from thousands of years ago and always play a vital role in curing diseases (Newman et al 2000). Plant derived bioactive natural products are potent herbal drugs to treat recent multi-drug resistant bacterial infections. The Genus *Cassia* belong to family leguminoseae (sub-family Casealpinaceae) comprises of about 500 species. Out of these 23 species occur in India (Pandit V. 2016). *Cassia* species are well known in folk medicine for their laxative and purgative activities along with other medicinal properties (Dalziel, 1956).

Cassia fistula L. and *Cassia javanica* L. are rich in metabolites viz; alkaloids, carbohydrates, flavanoids, triterpenoides and anthraquinone etc. In all secondary metabolites are present in whole parts of the plant have a strong tendency to contain anthraquinone glycosides. Anthraquinone glycosides are generally orange, red, brown-red compound. These are the famous for their laxative properties (Sakulpanich A. 2009). Many reports have shown that some *Cassia* species contain antibacterial, anti-diabetic, anti-malarial, anti-carcinogenic and hepatoprotective substances (Nanasombat el al., 2009; Tona1, 1999; El-Sawi, 2010: Sharma 2000). The aim of this study was examine anthraqinone glycosides in the leaves of targeted plant species for the estimation of anthraquinone glycosides and antimicrobial activity in methanolic extract to use the best antimicrobials for therapeutics and well being.

Materials and Methods

Plants Material

The fresh leaves of *C. fistula* and *C. javanica* were collected from CSIR- National Botanical Research Institute, Lucknow. The collected materials were authenticated and submitted in herbarium, CSIR- National Botanical Research Institute, Lucknow, India and their voucher specimen numbers are 102989 and 102990 respectively.

Extraction

The collected leaves were dried and powdered (40 mesh). The powder material was extracted by dissolving 2g into 100 ml of different polarity gradient solvents viz. hexane, chloroform, acetone, ethanol, methanol and water respectively for 24hrs (AOAC). The extract was then filtered, concentrated and vacuum dried.

Qualitative study of phytochemicals

Determination of functional group

C. fistula and *C. javanica* leaves were analyzed qualitatively to identify the presence of phytochemicals, viz. carbohydrates, alkaloids, anthraquinone, glycosides, tannin, flavanoids, phenolics, protein and terpenoids using the standard methods (Harborne J.B 1973, Kokate C.K., 1991, Trease and Evans, 1997) (Table 1).

Identification of Aloe emodin through Thin Layer Chromatography

Thin layer chromatography for identification of secondary metabolites/ Aloe-emodin was carried out in methanolic extracts. The fluorescent silica gel plate (silica gel $60F_{254}$ of Merck) was used & plates were made to run using solvents system, Ethyl acetate: Methanol: Water (10:1.35:0.9). The plates were observed in day light, short wavelength (UV-254 nm) & long wavelength (UV-366 nm), before derivatization. The Rf values of spots were calculated.

Quantitative analysis of anthraquinone glycosides content

0.3 g sample added in 30 ml of water and mixed. Weighed the mixture and refluxed for 15 minutes. Prepared aqueous mixture allowed to cool, weighed and adjusted to the original weight with water and centrifuged at 4000 rpm for 10 minutes. The residue and supernatant obtained. In 20 ml supernatant was added in 0.1 ml of 2M HCl. Extract was then washed three times with 15 ml chloroform, which results two layers. Chloroform layer discard and aqueous layer was used. 0.1 g $NHCO_3$ was added in aqueous layer, shake for 3 minutes and centrifuged at 4000 rpm for 20 minutes. 10 ml supernatant mixed with 20 ml of 10.5 % $FeCl_3.6H_2O$ then reflux for 20 minutes. 1 ml concentrated HCl was added and again reflux for 20 minutes. Then extract mixed with 25 ml ether for 3 times, after that aqueous layer discard and ether layer used. This layer was washed twice with 15 ml of water and then adjusted to 100 ml by ether. This ethereal extract divided into four equal volumes and

25 ml of extract was evaporated to dryness. The obtained residue was dissolved in 10 ml of 0.5 % methanolic magnesium acetate. This solution was used to record absorbance at 515nm. Total anthraquinone glycosides content was calculated as aloe emodin (mg/ml) using the following equation based on the calibration curve: $y= 9.81x + 0.198$, $r^2 = 0.997$, where y was the absorbance and x was the aloe emodin equivalent (mg/ml) (Standard of ASEAN herbal medicine 1993).

Test microorganisms

Standard strains of *Staphylococcus aureus, Salmonella typhyi, Escherichia coli and Pseudomonas aeruginosa* were obtained from microbiology Lab, CSIR National Botanical Research Institute, Lucknow India.

Antimicrobial activity

Antibacterial activity was determined using the agar disc diffusion method (Parekh J. 2005). Each bacterial inoculum was incubated in 2.5ml Mueller-Hinton broth at 37°C for 18 hours. Every inoculum was spread over plates containing Mueller-Hinton agar. Five millimeter discs containing 250µg/ml of methanolic extracts were placed on cultured pathogenic bacteria on agar plates and incubated at 37°C. The plates were checked for bacterial growth after a minimum of 16 hours and occasionally till 24 hours. The diameter of the zone of inhibition was then measured. Commercial disc of Streptomycin, Tetracycline and Itraconazole (30µg) was used as positive control.

Results and Discussion

Qualitative analysis

Results revealed that the secondary metabolites, viz; carbohydrates, anthraquinone, glycosides, alkaloids, triterpenoids, phenolics, flavanoids, proteins were present in different polarity gradient solvents shown in Table 1. In all extracts, hexane and chloroform have been minimum metabolites whereas methanolic extract has maximum metabolites carries. These phytochemicals may be a good source of medicinal application. Potential source of chemical marker viz; Aloe-emodin present in methanolic extract was identified by thin layer chromatography in (figure 1). The Rf value of chemical marker was calculated and compared with standard marker i.e. aloe-emodin (0.91). The methanolic extract of *C. fistula* leaf was found to be 0.91 and *C. javanica* leaf was 0.89 respectively.

A B C

Figure -1. A- *C. fistula* leaf, **B-** *C. javanica* leaf, **C-** Chemical marker

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Table 1. Phytochemical screening in different extracts for functional group testing

Metabolites	Solvent extracts of *Cassia fistula* (*C.f.L*) and *Cassia javanica* (*C.j.L*) leaves											
	Hexane		Chloroform		Acetone		Ethanol		Methanol		Water	
	C.f.L	C.j.L	C.f.L	C.j.L	C.f.L	C.j.L	C.f.L	C.j.L	C.f.L	C.j.L	C.f.L	C.j.L
Carbohydrates	+	-	-	-	-	-	++	++	-	-	+++	+++
Anthraquinone	-	-	-	-	-	-	+	+	++	+++	+	+
Glycosides	-	-	-	-	+	+	++	++	+++	+++	++	++
Phenolics	-	-	-	-	+	+	+	+	++	++	-	-
Alkaloids	-	-	-	-	+	+	+	+	++	++	-	-
Flavanoids	-	-	-	-	+	+	+	+	++	++	-	-
Protein	-	-	-	-	-	-	+	+	+	+	++	++
Tri-terpenoids & sterol	+	+	+	-	+++	+++	++	++	++	++	+	+

(+) = Present
(-) = Absent

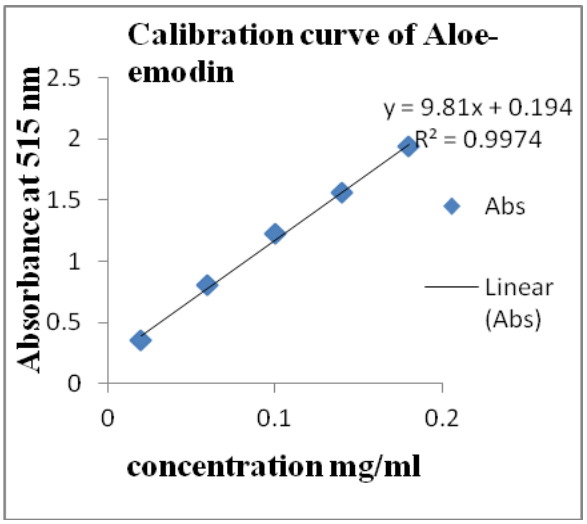

Figure- 2

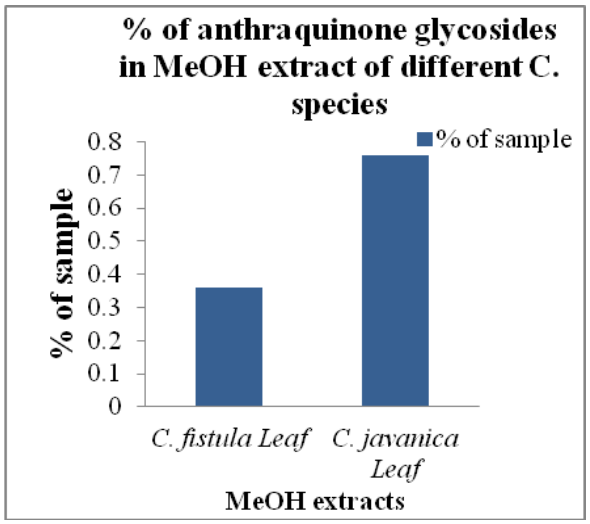

Figure- 3

Quantitative analysis

In quantitative estimation of anthraquinone content in methanolic extracts of both *Cassia* species using Aloe- emodin as standard (Fig 2). Aloe- emodin content was 0.36% in *Cassia fistula* leaf whereas 0.76% was present in *Cassia javanica* leaf respectively (fig. 3). The methanolic extract of *C. javanica* leaves has maximum percentage as compared with *C. fistula* leaves. Anthraquinone is major secondary metabolites found in *Cassia* species and highly potential chemical marker use in pharmaceutical industry for further pharmacological activity.

Antimicrobial activity

The result of antimicrobial activity in methanolic extract of *C. fistula* and *C. javanica* leaf against test microorganism are summarized in Table 2. The zone of inhibition was calculated in mm of the extract and three antibiotics are used. The methanolic

extract of *C. fistula* leaf showed the highest antimicrobial activity against *Staphylococcus sp.* (14 mm) followed by *Salmonella sp*. (13 mm), *E. coli* (12 mm) and *Candida sp.* (10 mm). In *C. javanica* leaf extract was found to be highest activity showed against *Staphylococcus sp.* (13 mm) followed by *E. coli* (12 mm) whereas *Salmonella sp.* and *Candida sp.* were found (10 mm). On the other hand *C. fistula* exhibit much better antimicrobial activity as compared with *C. javanica* leaf against all microbial strains (Figure 2). The streptomycin was more susceptible to *Staphylococcus sp.* and *Salmonella sp*. as compared with other antibiotics. *C. fistula* could be a source of most promising future antimicrobials as plant based natural products.

Table 2. Inhibition zone (mm) of Methanolic extract of different *C. species* leaves against test microorganism

Microorganism	Streptomycin (mm)	Tetracyclin (mm)	Itraconazole (mm)	*C. fistula* leaf (mm)	*C. javanica* leaf (mm)
Staphylococcus sp.	35	30	-	14	13
Salmonella sp.	35	32	-	13	10
E.coli	34	27	-	12	12
Candida sp.	-	-	16	10	10

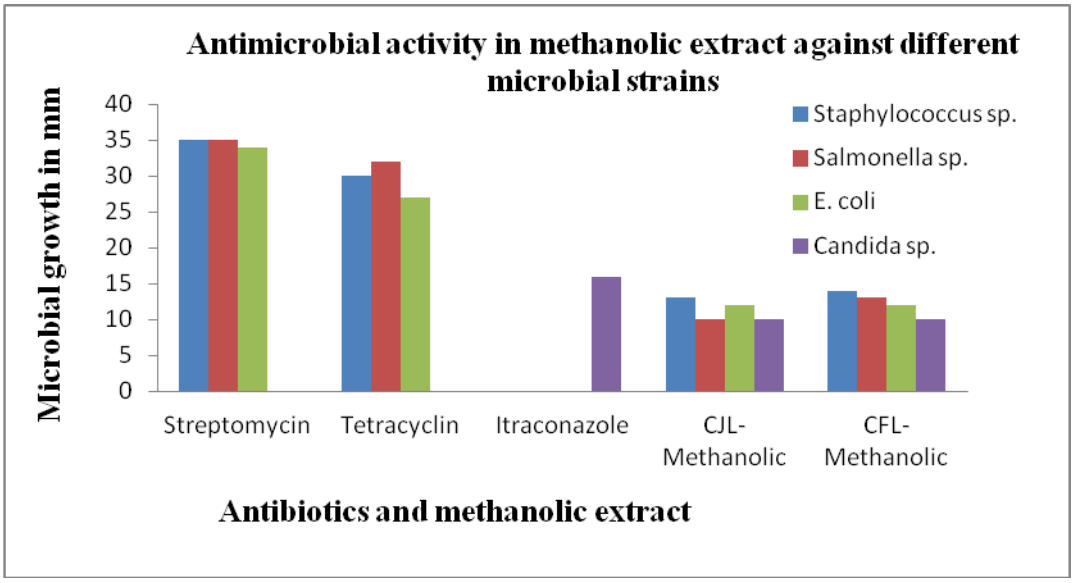

Figure 4. Antimicrobial activity in different *C. species*

Conflict of interest statement

The authors declare there is no conflict of interest.

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Review Article

ROLE OF BOTANICALS IN PLANT DISEASE MANAGEMENT – A REVIEW

Vijayshree Gahlot and Mohit Kumar

Department of Plant Pathology, College of Agriculture,
Swami Keshwanand Rajasthan Agriculture University, Bikaner, Rajasthan
*Corresponding author: vijayshree1789@gmail.com

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Abstract

Plant diseases contribute significantly to the total crop losses both at global and national level. In order to mitigate these losses, pesticides are being used. Discovery of Bordeaux mixture is significant in the history of chemical control of plant diseases. The use of synthetic pesticides - fungicides - has undoubtedly resulted in increased crop production. However, these chemicals are hazardous both to man and the environment. Furthermore, they cannot be afforded by many farmers in India, especially with the removal of subsidies on agricultural inputs. To circumvent this situation, new locally produced and safer alternative pesticides should be made available. Research has been done worldwide on the use of botanical pesticides in plant disease control and extracts from many plant species have been found to be active against many phytopathogenic fungi. Further work is required to determine the potential of these extracts in vivo. Some botanicals and the plant pathogens against which they are active are discussed in this review.

Keywords: Botanicals, plant pathogen and pesticides

Introduction

The ultimate aim of recent research in this area has been the development of alternative control strategies to reduce dependency on synthetic fungicides. Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the pathogen. These groups of compounds show antimicrobial effect and serves as plant defence mechanisms against pathogenic microorganisms The volatile antimicrobial substance allicin (diallyl thio sulphonate) is synthesized in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine Sulphoxide) mixes with the enzyme alliin-lyase. Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. Allicin effectively controlled seed-borne Alternaria spp. in carrot, Phytophthora leaf blight of tomato and tuber blight of potato as well as Magnaporthe on rice and downy mildew of Arabidopsis thaliana. Application of plant products especially essential oils is a very attractive method for controlling post harvest diseases. Essential oil extracted from lemon grass (Cymbopogon spp.) post harvest anthracnose of mango fruit. The anti viral protein (AVP) extracts from Bougainvillea spectabilis and Prosopis chilinesis were found to be effective in reducing the sunflower necrosis virus

(SFNV) infection both in cowpea and sunflower plants . At present, scientists are investigating for plant products of antimicrobial properties. It . be advantageous to standardize methods of extraction and in vitro antimicrobial efficacy testing so that the search for new biologically active plant products could be more systematic. Thousands of phyto chemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected in vivo testing to evaluate the efficacy in controlling the incidence of diseases in crops, plants, and humans.

Botanicals

Some plant contains components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components are called botanical pesticides or botanicals.

Commonly used botanicals in plant disease management are given below:

Plant extracts

Neem (*Azadirachta indica*, A. Juss), Garlic (*Allium sativum*, Linn., Eucalyptus (*Eucalyptus globulus*, Labill., Turmeric (*Curcuma Longa*, Linn); Tobacco (*Nicotiana tabacum*, Linn., Ginger (*Zingiber officinale*, Rosc.) Onion (*Allium sativum*)

Essential oils

Nettle oil (*Urtica* spp.), Thyme oil (*Thymus vulgaris*, Linn.), Eucalyptus oil *Eucalyptus globulus*, Labill. Rue oil (*Ruta graveolens*, Linn.), Lemon grass oil (*Cymbopogon flexuosus* (Steud.) Wats. and Tea tree oil (*Melaleuca alternifolia).*

Advantages of botanicals

1. Sustainable solution of plant disease management
2. Reduce crop losses
3. Eco-friendly
4. Easily bio-degradable Organic farming
5. Cheaper
6. Integrated Diseases Management

Fungitoxic botanicals

Natural products with pesticidal activity have been and are being explored in order to make available pesticides which are easily biodegradable, selective and which can be locally produced, especially for farmers who cannot afford expensive synthetic pesticides. At present serious attention is drawn to extracts from higher plants known to contain antifungal substances in the form of alkaloids or prohibitins, which help in resisting the pathogens.

Bhowmick and Vardhan (1982) have evaluated the antimycotic activity of leaf extracts of some medicinal plants on *Dreschslera turcica* (pars) and observed that extracts from Vitex negundo and *Catharanthus roseus c*an completely inhibit the growth of the fungus in vitro.
Five antifungal substances that show activity against the Japanese pear pathotype of *Alternaria alternata* in vitro have been isolated from extract of *Portulaca oleracea* L. These antifungal substances are isobutyric acid, butyric acid, isovaleric acid, valeric acid and caproic acid (Park *et al*., 1986). *Alternaria alternata* and *Fusarium oxysporum* are known to be inhibited by extracts of young and mature leaves of *Codieum variegatum* with the extracts of young leaves being more active against A. alternata and the old leaves against *F. oxysporum (*Naidu, *1988).* Neem *(Azadirachta indica)* oil preparations are reported to have inhibited growth of A. alternata by 61.1 and 100% at a concentration of i and 10% respectively (Dharam and Sharma, 1985). *Ryania speciosa*, family Flocourtiaceae, is prevalent in Northern parts of South America and the Amazon basin. Dried roots and leaves when finely pounded are known to be effective against maize smut and stalkborers (Stoll, 1986)

Natarajan and Lalithakumari; (1987). In vivo spraying rice leaves with extracts from *Lawsonia inermis* is reported to give better control of *Dreschslera oryzae* than seed treatment

Manoharachary and Gourinath (1988) have determined the efficacy of some tropical plant extracts against four pathogenic fungi. Plant extracts from roots, stems, leaves, flowers and fruits of one hundred plants belonging to 37 families were screened for fungitoxicity against *Curvularia lunata* (Wakker) Boedijn, *Cylindrocarpon lichenicola* (C. maassal) Hawksworth, *Fusarium solani* (Mart sacc.) and *Myrothecium leucotrichum* (peck) Tulloch. The effect of the plant water extracts was evaluated by using a spore germination test which involved an evaluation of growth and sporulation of the fungi. Plants tested included Calatropis, Datura, Ocimum, Ricinus and Thidax. Among the plant parts tested, extracts of roots and flowers were found to be more effective followed by leaf extracts. The performance of these extracts in vivo has not been investigated.

Bandara and his colleagues (1988, 1989) at the University of Peradeniya in Sri Lanka evaluated three rhizomatous perennial herbs used in native medicine for their antifungal and antibacterial properties. The herbs tested were: *Acorns calamus (Araceae), Zingiber zerumbet* and *Curcuma longa (Zingiberaceae).* Crude extracts of rhizomes at various dilutions were evaluated for their effect on growth and sporulation of *Cladosporium sp., Botryodiplodia theobromae, Fusarium solani, Phytophthora infestans, Pythium sp.* and *Pyricularia oryzae.* Their inhibitory action was compared to that of Benlate. Their findings revealed that extracts of *A. calamus* and *Z. zerumbet* had profound effect on growth of all fungi tested. It was particularly observed that the inhibition of growth of F. solani was significantly higher in *A. calamus* extract than Benlate. Sporulation of *B. theobromae, F. solani, P. oryzae* was also inhibited.

Bandara *et al.* (1989) have screened furthermore 36 medically used plant species in Sri Lanka for their activity against *Cladosporium cladosporioides*. Plant species whose extracts displayed significant activity were, *Buteamonosperma*(stem bark), *Costus specious* (rhizome*), Curcuma zedoaria* (tuber), *Eupatorium riparium* (whole plant, root), Pleisospermium alatum (stembark, rootbark) and Z. zerumbet (tuber). The active constituent of C. specious has been isolated and identified as methyl 3 - (4-hydroxyphenyl)-2 (E)-propenoate. The compound's activity against *Penicillium sp. Aspergillus niger, C. cladosporioides* and *Curvularia sp.* has been evaluated and its activity against the two latter fungi has been comparable to that of the standard fungicide Benlate.

Mishra *et al*. (1989), have isolated essential oils from leaves of *Chenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum* and *O. grattissium.* These oils have demonstrated fungitoxicity against Aspergillus flavus at 200, 300, 400 and 500 ppm and most of them have shown to be more effective than synthetic fungicides *viz*; Agrosan G.N., Copperoxychloride, Ceresan, Thiovit and Dithane M45. Asthana *et al.* (1986) have found the leaf extract of *Ocimum adscendens* to be fungitoxic against *Aspergillus flavus.* The volatile fungitoxic fraction has been identified to be an essential oil, and has been observed to be more active than some five synthetic fungicides tested.

Alternaria leaf blight is one of the major diseases of pigeon pea (*Cajanus cajan*) an important legume which is a major source of protein to the predominantly vegetarian diet of Indian people. From *Tiliacora racemosa*, an antidote to snake bite in Indian medicines, two alkaloids have been isolated and

evaluated for their antifungal activity against *Alternaria termissina*. Tiliacorine reduced the germination of the fungus at concentrations > 100 ppm (Tripathi and Dwivedi 1989). Tiliacorine is translocated symplastically in plant tissue and thus works as a systemic fungicide (Singh and Pandey, 1988).

Adeleye and Ikotun (1989) of the Department of Agricultural Botany at the University of Ibadan have found that the extracts from a wild variety of *Dioscorea bulbifera* L. has some antifungal activity against 5 plant pathogenic fungi namely: *Sclerotium rolfsii, Curvularia lunata, Fusarium moniliforme, Macrophomina phaseolina* and *Botryodiplodia theobromae*. The alkaloid dihydrodioscorine isolated from the extract was tested against the fungi in vitro by incorporating it in PDA at 0.1% concentration in its HCl form. Noriel and Robles, (1990). *Helminthosporium maydis* Nisik, causes leaf spot disease in maize. It occurs worldwide and have become important in regions of warm (20-32°C), damp climate. Extracts of *Portulaca oleracea* L. have been found to possess protective and therapeutic activities against this disease Wang *et al.* (1990) have been able to isolate antifungal and larvicidal polyacetylenes for *Artemisia borealis (B. campestris* subsp. borealis). Dichloromethane extracts for the whole plant have shown antifungal activity against *Cladosporium cucumerinum.* Work by Upadhyaya and Gupta (1990) have demonstrated the inhibitory effect of some medicinal plants on the growth of *Curvularia lunata* (*Cochliobolus lunatus).* Ethanol extracts of garlic followed by those of Ocimum santum, Datura alba and hemp were found to be most inhibitory to growth of the fungus. Aqueous extracts were less effective. Garlic extracts have shown to be inhibitory on the growth of a number of fungi (Tansey and Appleton, 1975).

From methanol extracts of twigs of Oxymitra velutina - a west african plant, 12 alkaloids; 5 aporphinoids including lysicamine, which is active against *Bacillus subtilis, Botrytis cinerea, Saprolegnia asterophora* and *Rhizoctonia solani,* have been isolated (Achenbach and Hemrich, 1991).

Yegen *et al.* (1992) have studied the fungitoxic effect of extracts of six selected plants from Turkey. Results indicate that aqueous and essential oils of *Thymbra spicata, Satureja thymbra, Laura nobilis, Mentha spicata, Salvia futicasa* and *Inula viscosa* are fungitoxic to *Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Phytophthora capsici.* Kumar and Tripathi (1991) have screened leaf extracts of 18 plant species belonging to 11 families for their control of *Pythium debaryanum, Fusarium oxysporum, R. solani* and *Sclerotium rolfsii.*

Conclusion

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property . The ethno- botanical study of plant is important for modern day medicine but its usefulness cannot be overemphasized if methods are not standardized to obtain comparable and reproducible results. At present, scientists are investigating for plant products of antimicrobial properties. It would be advantageous to standardize methods of ex-traction and in vitro antimicrobial efficacy testing so that the search for new biologically active plant products could be more systematic and interpretation of results would be facilitated. Thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected in vivo testing to evaluate the efficacy in controlling the incidence of

disease in crops, plants, and humans. Efficient collaborations with pharmacologists and medical doctors, plant pathologists and microbiologists are crucial to see the complete development of an interesting lead compound into an exploitable product. Most of the food crops here in India are grown almost exclusively by small-scale farmers and thus any significant increase in food production can only be achieved by this group of producers (Brader, 1992). Efforts must be made to assist these farmers and one way of doing this is to make available less hazardous pesticides which they can afford

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