IJPSR (2017), Vol. 8, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 August, 2016; received in revised form, 19 October, 2016; accepted, 06 December, 2016; published 01 March, 2017

ANTIMYCOTICS IN CERTAIN TAXA OF EUPHORBIACEAE

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Keywords:

Survey of Antimycotics in Euphorbiaceae

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ABSTRACT: There is a lot of thrust on utilization of bioactive chemicals from plant systems and their significant effect on microorganisms. Ten species of plants of the family Euphorbiaceae have been used for our study they are, *Croton bonpandianum, Euphorbia tirucalli, E. hirta, E. heterophylla, Jatropha curcas, J. glandulifera, J. multifida, Phyllanthus amarus, Ricinus cummunis, Pedilanthus tithymaloides*. Most of the family members of Euphorbiaceae have unique feature of possessing latex in their plant body. This latex is a secondary metabolite and hence has no significant physiological role in the plant system. An attempt has been made to find whether the plant extracts prepared in water have any role against fungi. These fungi are pathogenic to both plants and animals. The stem and leaves are thoroughly washed in 1% mercuric chloride and later washed in tap water followed by double distilled water. Later on crushed in mortar and pestle using water. The fungal species used for our study are *Aspergillus niger*, *Saccharomyces cerevisiae* and *Fusarium oxysporum.* The extracts are directly used for their antimycotic activity. Most of the plant extracts of Euphorbiaceae showed inhibition of growth of the fungi but for few exceptions. The study envisages to find new biodegradable pesticides, presently which are necessary to control plant diseases.

INTRODUCTION: The use of plant or plant parts as medicines is known since ages. The Indian medical systems and various other systems of medicines practiced elsewhere in the world, used plants for over several centuries. These traditional systems of medicines have served 70% of the rural and tribal folk of developing countries for a variety of diseases Gangoue-Pieboji *et al* [1]. All systems of medicines including Homeopathy, Allopathy, Ayurveda, Siddha and Unani have used plants to prepare drugs, according to the World Health Organization Report Santo *et al* [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(3).1229-34

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (3).1229-34

As per 'the plantlist' web site over 3,50,699 plant species have valid names, which are existing or non existing on this earth. Among the existing plants, only 2% of plants are explored and tested for their antioxidant activity Mahuya *et al* [3]. In recent years, the medicinal species that reside in natural areas have received increased scientific and commercial attention, worldwide. There are as many as 50,000-80,000 flowering plants used medicinally, IUCN species survival commission [4], Marinelli [5].

The plants are rich in bioactive compounds and used to cure a wide range of ailments, in the form of crude preparations in Ayurveda and Siddha. These bioactive compounds are concentrated in various parts of the plant like root, stem, leaf, flower, fruit and seed. It is also known these bioactive compounds are present at a specific part of the day.

The majority of plants have antibiotic activity and also possess antioxidant free radicals. Generally the antioxidants have medicinal value because the cell damage is reduced by nullifying the effect of free radicals in the human body [3].

The plants that are investigated belong to the family Euphorbiaceae. The Euphorbiaceae is one of the sixth largest families among the Angiosperms. The members of the family Euphorbiaceae are laticiferous and produce many types of secondary metabolites like polycyclic triterpenes, flavonoids, curcin and cyclic heptapeptides etc. Additionally, the members possess characteristic extra floral nectarines on stem, petiole, leaf and peduncle Gopalakrishna Bhat [6].

During, the last few decades there was thrust on finding new antibiotics from plant sources. When compared to antibacterial drugs, in antimycotics less work has been done and hence an attempt to screen ten different taxa of Euphorbiaceae against fungi such as; *Aspergillus niger, Saccharomyces cerevisiae* and *Fusarium oxysporum* Iwu *et al* [7], Chopra *et al* [8].

The earliest record of Ethanopharmacology goes back to the period of Ayurvedic classic work like Charaka Samhita (1000 BC), who mentioned the use of 2000 herbs for the preparation of Ayurvedic medicines. Charaka Samhita along with Sushruta Samhita the most ancient and authoritative treatises of Ayurveda. Charaka Samhita and Sushruta Samhita now identified all over the world as an important early source of medical understanding and practice. The attempt of plant extracts against plant pathogens was tried by Bhargava *et al* [9] have proved fungistatic effect of *Ocimum cannum* L. Bhatnagar and McCormick [10] have shown the inhibitory effect of Neem leaf extracts against aflatoxin synthesis in *Aspergillus parasiticus* and Bhatnagar *et al* [11] have shown inhibition effect of Neem leaf extracts against aflatoxin synthesis in *Aspergillus flavus* and *A. parasiticus*. Similarly Al-Abed *et al* [12] have studied the antifungal effects of some common wild plant species and Hoffman *et al* [13] screening of ten medicinal plants for antibacterial and antifungal activity.

Aspergillus: produces mycotoxin-ochratoxin, isoflavone, orobol, fumonisin B2 and aflatoxins. The species of *Aspergillus* causing various diseases in plants and animals are black mold in onions, peanuts and grapes. The spoilage of kernels and fruits in cashew, dates, figs and Vanilla and of root curling and crown rot in peanut is because of *Aspergillus* species. Among humans it causes lung disease called aspergillosis and ear infections called otomycosis. Rangswami and Mahadevan [14] and Aggarval and Mehrotra [15].

Fusarium: Produces wilt disease in banana, cotton, sweet potato, tomato, ginger, asperagus and muskmelon. Fungal keratitis, onychomysis, hyalohypomycosis and cutaneous infections in humans and animals is caused by Fusarium Rangswami and Mahadevan [14] and Aggarval and Mehrotra [15].

Saccharomyces cerevisiae: Causes a disease in humans called fugemia Rangswami and Mahadevan [14] and Aggarval and Mehrotra [15].

2. MATERIALS AND METHODS:

2.1 Plant materials of Euphorbiaceae screened for antimycotics:

1. *Croton bonplandianum Baill.:* Herb, stem stellately hairy. Leaves crenate-serrate 3-5 ribbed from base often glandular at base. Flowers monoecious. Female flowers have 1-whorl of perianth and male flowers have petals and sepals. Stamens more than 10, Styles -3, with bifid stigma Rastogi and Mehrotra [16].

2. *Euphorbia tirucalli* Linn.: The trunk and branches of aged plants are woody, younger branches are green and cylindrical looks like pencils and hence 'pencil plant'. The leaves are insignificant and minute. The plant is also called 'petroleum plant' because it produces hydrocarbon substances very much like gasoline.

The latex of stem and branches is used for skin cancer and syphilis ulcers. The decoction of the branches and leaves used for colic and gastric problems Jigna Parekh *et al* [17].

3. *Euphorbia hirta* Linn.: A small erect ascending annual herb reaching upto 50 cms with hairy stems. The flowers are small, crowded together in dense cymes and enclosed in a cup like involucre called cyathium.

The plant is used as antiamoebic, antispasmodic, anti-inflammatory. Characteristically the plant is antibacterial and anticancerous. Jigna Parekh *et al* [17].

4. *Euphorbia heterophylla* Linn.: Annual herbs growing to the height of 0.25 m to 0.75 m. Flowers are reduced and enclosed in an involucre of bracts referred as cyathium. The involucral cup enclose large number of male flowers in the periphery and with a single female flower at the centre. The cup in its periphery possess nectar gland. The synonym of *E. heterophylla* is *E. glandulifera* Meenakshi Sundaram *et al* [18]. The plants are used as insect repellant. The stem extract has wound healing effect. Consuming 1-2 leaves every morning helps for easy passage of bowels.

5. *Jatropha curcas* Linn.: A large deciduous soft wooded shrub. Leaves broadly ovate, palmately 5-lobed. Flowers in axillary cymose panicles. Male flowers- corolla villous within longer than calyx. Female flowers- petals, rarely exceed the sepals in length. Fruits ovoid breaking up into trivalved cocci.

The seed oil is used as biodiesel. The alkaloid contains anticancerous property. The young twigs are used to brush teeth. Roots have antidote for snake poison. Jatropha oil cake is rich in organic content Aswani Kumar and Satywati and Sharma [19].

6. *Jatropha glandulifera* Linn.: The plant is a shrub or under shrub. The petioles have hairy structures with a poison gland at tip. Leaf blade palmately trifid with glandular hairs along the margin.

The root extract prepared in water reduce abdominal enlargement. The leaves are used in skin disease Surendra *et al* [20].

7. *Jatropha multifida* Linn.: A cultivated ornamental small shrub, 1-2 m in height. Leaves pinnately cut into 9-11 cm deep, narrow–lobes. Flowers small, scarlet, with small yellow petals, produced in clusters in the axils of leaves. The seeds have been used as purgative, antihelminthic, and abortifacient. The seed oil are used in rheumatic pains Surendra *et al* [20].

8. *Phyllanthus amarus Schumac. and Thonn:* A herb of 10-60 mm tall, erect, stem terete. Leaves elliptic, oblong-obovate. Flowers axillary, 1-3 male flowers. Stamens -3, connate. Female flowers pedicellate. Styles-3. Each is shallowly bifid at apex.

Leaves and stem parts is used in jaundice, diarrohoea. This is antiviral and antihepatotoxic Patel *et al* [21].

9. *Ricinus cummunis Linn.*: A large shrub sometimes appear like a small tree. Leaves biglandular at tip, leaf blade palmately 7-fid. In male flowers perianth 3-partite and in female flowers styles-3 with 6-papillous arms.Fruit densely muricate.

Castor is cultivated sometimes wild, growing in waste places. The oil is extracted from seeds, used to produce high grade lubricant. Sometimes the oil is hydrogenated to produce waxes, polishes, candles and crayons. Castor oil is used in Ayurvedic medicinal preparations Yadav *et al* [22].

10. *Pedilanthus tithymaloides (Linn.) Poit.*: A cultivated fleshy shrub grow upto 1m high. Leaves simple, alternate 4-10 cm long. Flowers in cyathia borne in terminal clusters. Cyathium cup is made of 2-lipped red bracts, 8-12 mm long with a saclike base. It appears like a lady's slipper.

Used as insect repellant, antiseptic and antihelminthic Soma Ghosh *et al* [23].

2.2 Preparation of Plant extracts: The extracts of leaf and stem are prepared of the above mentioned plants. The fresh stem and leaf parts are cut into small pieces washed with tap water and surface sterilized with 1% mercuric chloride and again washed thoroughly in running double distilled water for about 30 minutes to avoid any traces of mercuric chloride residues. The plant parts are crushed in pestle and mortar, later the extract was strained through cheese cloth and collected in sterilized test tubes. These extracts are used for our experiments against fungi like *Aspergillus niger* (MTCC No 1344), Saccharomyces cerevisiae (MTCC No 170) and *Fusarium oxysporum* (MTCC No 284) which are maintained as pure cultures in the laboratory of Microbiology.

3. RESULTS AND DISCUSSION: The plant species examined, have shown inhibition effect on *Aspergillus niger* and *Saccharomyces cerevisiae* and of *Fusarium oxysporum* species. The inhibition zone in different plant extracts is significantly varied (**Table 1, Fig. 1**).

In species of *Aspergillus niger* there was maximum inhibition zone of 1.85 cm diameter is observed in a plant extract of *E. tirucalli* and least inhibition zone of 0.25 cm is observed in in *E. heterophylla*, while there is no inhibiton in species of *E. hirta, Jatropha curcas, J. glandulifera and Phyllanthus amarus*, and of *P. amarus* is being used as a popular medicine for jaundice. The inhibition of *Aspergillus niger* and of *Saccharomyces cerevisiae* almost similar by plant extracts of *Croton bonplandianum* and *Pedilanthus tithymaloides* (**Table 1, Fig. 1, 2**).

TABLE 1: INHIBITION ZONE IN DIFFERENT LEAF AND STEM EXTRACTS

Names of Plant species	*Aspergillus niger*	*Saccharomyces cerevisiae*	*Fusarium oxysporum*
Croton bonplandianum	1.2 cm	1.6 cm	-ve
Euphorbia tirucalli	1.85 cm	1.2 cm	-ve
Euphorbia heterophylla	0.25 cm	1.35 cm	-ve
Euphorbia hirta	-ve	-ve	-ve
Jatropha curcas	-ve	1.6 cm	-ve
Jatropha glandulifera	-ve	-ve	-ve
Jatropha multifida	1.7 cm	2.1 cm	-ve
Phyllanthus amarus	-ve	1.0 cm	-ve
Ricinus communis	1.3 cm	2.0 cm	-ve
Pedilanthus tithymaloides	1.2 cm	1.8 cm	0.5 cm

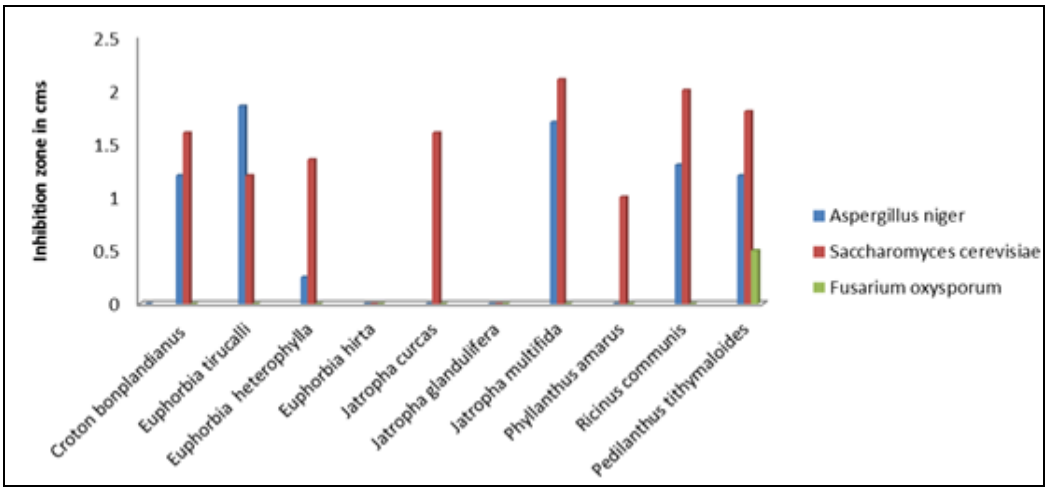

FIG. 1: COMPARATIVE INHIBITION ZONE OF MICROBES (FUNGI) IN DIFFERENT SPECIES OF LEAF AND STEM EXTRACTS

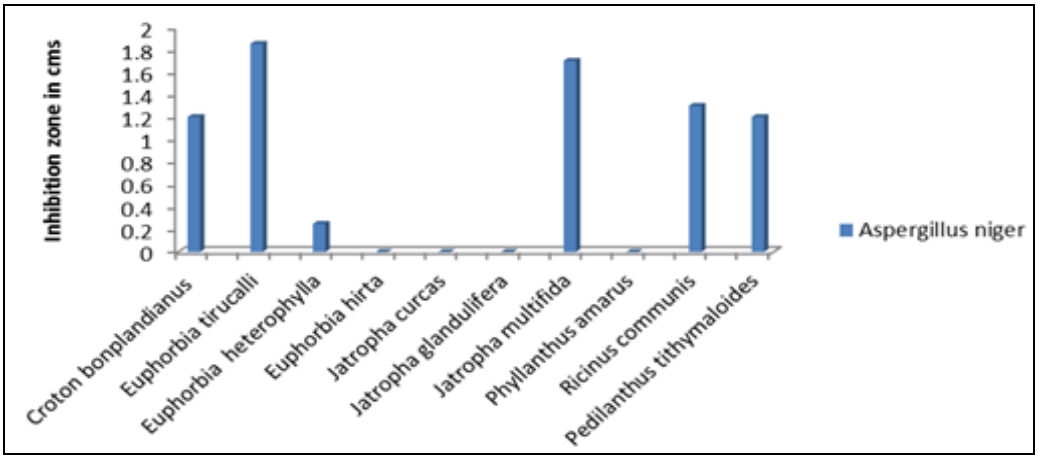

FIG. 2: INHIBITION ZONE IN *ASPERGILLUS NIGER*

In species of *Saccharomyces cerevisiae* the maximum inhibition zone of 2.0 cm and 2.1 cm diameter is observed in plant extracts of *Ricinus communis*, and *Jatropha multifida* respectively and the least inhibition in *Phyllanthus amarus*, and no inhibition in *Euphorbia hirta* and *Jatropha glandulifera*. The response to the other plant extracts is of more than 1 cm(**Table 1, Fig. 1, 3).**

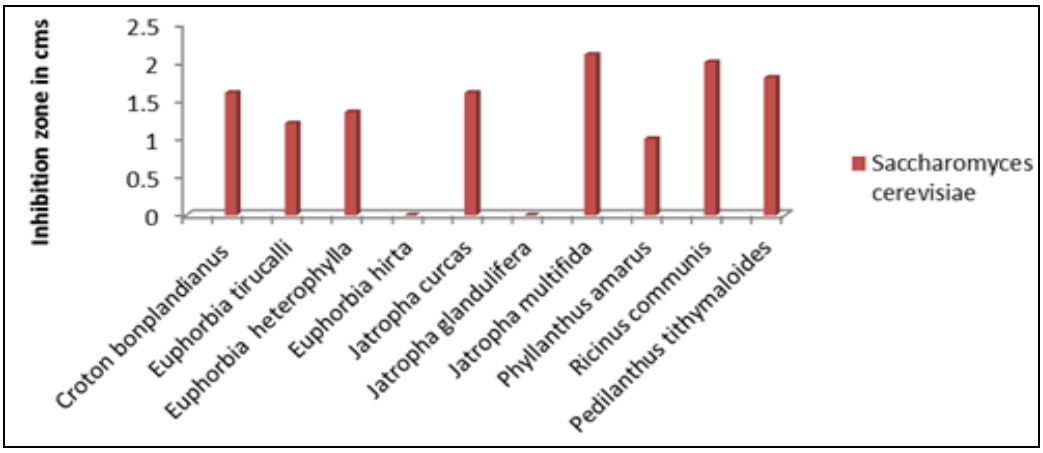

FIG. 3: INHIBITION ZONE OF *SACCHAROMYCES CEREVISIAE*

In *Fusarium oxysporum* the inhibition effect on *Pedilanthus tithymaloides* is of 0.5 cm but in all others negative result is observed. However, there was no growth of mycelium around the plant extract pit **(Table 1, Fig.1,4**).

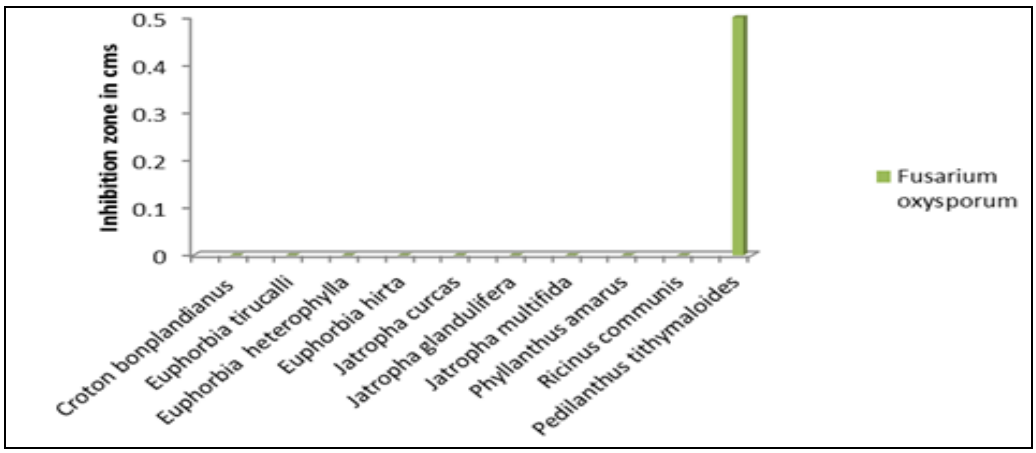

FIG. 4: INHIBITION ZONE OF *FUSARIUM OXYSPORUM*

CONCLUSIONS: Most of the species of Euphorbiaceae used in this study have medicinal value. The observations have revealed that the most of the members possess antifungal activity against the species of *Aspergillus niger*. While the plant extracts of *Euphorbia hirta* and *Jatropha glandulifera* have not shown any kind of inhibiton in the growth of *Saccharomyces cerevisiae*.

Euphorbia hirta, Jatropha curcas J. glandulifera and Phyllanthus amarus showed no inhibition zone against *Aspergillus niger*. The maximum antifungal activity against *Aspergillus niger* is noted in *Euphorbia tirucalli* and least antifungal activity was noted in *E. heterophylla*. Similarly, the species of *Saccharomyces cerevisiae* is more susceptible to all the investigated species except *Euphorbia hirta* and *Jatropha glandulifera*.

All the test fungal species are pathogenic in both plants and animals. These plant extracts are significantly pollution free compared to chemical pesticides used in crop plants to control pests. The extracts are natural products and are bio-degradable. More so the plant extracts are cost effective. Further, the work can be carried out to study, their chemical components and the mechanism of their inhibition effect of the fungi.

This would enhance the use of biodegradable products as pesticides to prevent diseases in plant crops.

ACKNOWLEDGEMENT: The authors are thankful to Biological Science Department, Bangalore University and Indian Academy Degree College to provide an opportunity to conduct the above research.

CONFLICT OF INTEREST: The authors report no conflict of interest.

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How to cite this article:
Vimala R, Mrunalini BR, Ghosh MD and Ramakrishna TM: Antimycotics in certain taxa of euphorbiaceae. Int J Pharm Sci Res 2017; 8(3): 1229-34.doi: 10.13040/IJPSR.0975-8232.8(3).1229-34.