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Evaluation of Antibacterial and Antifungal Activity of *Moringa Concanensis*

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ABSTRACT

In today's scenario, fungal and bacterial infections are one of the most challenging pestilential diseases for the health-care professional which is increasing with a very high rate in the growing population, especially among the immune compromised people.The present work involved the study to extract active principles of *Moringa concanensis* and perform antibacterial and antifungal activity for various extracts. The leaves were dried powdered unexpected using solvents petroleum ether, chloroform, ethanol and water. Chemical tests were performed to ascertain presence of various classes of phyto-constituents like alkaloids, glycosides, saponins, carbohydrates, proteins, amino acids tannins, flavonoids and phenolic substances. All four extracts showed the presence of various phytochemicals responsible for multiple pharmacological activities. The extracts were reconstituted using 1% aqueous solution of carboxy methyl cellulose and were tested for antibacterial and antifungal activity against six microorganisms, namely *Bacillus subtilis, Enterobacter aerogenes, Bacillus cereus, Aspergillus Flavus, Aspergillus Niger and Helminthosporium*, using well diffusion method. The results of the activity are promising with chloroform extract showing highest inhibitory activity against most of the organisms. All the extracts were able to demonstrate significant activity compared to control inhibition of microorganisms tested and hold key to discover lead molecules after further scientific investigation.

KEYWORDS: Antibacterial; Antifungal; *Moringa concanensis*; Phytoconstituents; Immunocompromised.

Introduction

Herbal plants are widely used as folk medicine to treat large number of diseases (Sachan et al., 2020). Herbal drugs play an important role in health care program especially in developing countries (Das et al., 2016). Herbal medicinal drug may be single active constituent or entire herb source is considered as medicinal product (Sachanamd Kumar, 2015). Bio molecules in the plants play a crucial role in health maintenance and promotion (Sachan et al., 2018). Now-a-days, individuals highly depend on allopathic medicines rather than the ayurvedic, whereas in the primal days people depend on natural remedies for the treatment of different diseases.

Herbal medicines also referred as phytomedicines allude to the utilization of any plants, roots, flowers, seeds, bark, and leaves for therapeutic purposes. These are standardized herbal complex mixtures of at least one plant which are utilized in many nations for the cure of different diseases. There are various herbal products accessible that claim to treat an extensive variety of issues from sorrow to cold and flu.

Often these are part of traditional medicine and often these are not evaluated using pharmacological principles and experimental model. Although there is claim that other medicines are completely safe still there are chances of them showing unwarranted effect and is also needed to be evaluated. It does leave a lot of gap between the knowledge we have from traditional systems of medicine and its correlation with the modern medicine (Rao et al., 2020).

Moringa species is an easily and fastly developing soft wood tree and it reaches nearly the maximum height of 12m and is aboriginal to the Himalayan foothills. The bioactive compounds of Moringa leaves are grouped as vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, oxalates and phytates (Leone et al., 2015).

The foliage, flowers and immature pods (fruits) of numerous commercially grown Indian cultivars of Moringa have been characterized by the content of carotenoids. All-E-lutein is the major carotenoid in foliage and immature pods (fruits), accounting for 53.6 and 52.0% of the total carotenoids respectively. Immature pods and flowers are characterized by a higher content of total monounsaturated fatty acids (MUFAs, 16–30%) and are low in PUFAs (34–47%) compared to the leaves (Abarikwn et al., 2017).

During the past centuries, worldwide traditional healers have applied various parts of Moringa tree as traditional medicine. In Moringa sp. like the roots, leaves, flowers, fruits and seeds are well known to be very rich sources of phytochemical 17 compounds (Saadabi & Abuzaid, 2011). It has already been described that *M. oleifera* contains alkaloids, carotenoids, tannins, anthraquinones, anthocyanins and proanthocyanidns (Sinha et al., 2011).

Materials and Methods

Plant Material

The leaves of *Moringa concanensis* Nimmo. were collected from the local market of Lucknow, Uttar Pradesh and authenticated from department of Pharmacy, Integral University. The voucher specimen IU/ PHAR/HRB/19/15 deposited in the PG and Research Department of Integral University, Lucknow, Uttar Pradesh, India. The leaves of plants were washed thoroughly 2-3 times with tap water and sterilized by spraying with 70% alcohol and air dried at room temperature.

Preparation of Plant Extracts

Air dried powdered samples of M. concanensis Nimmo. Leaves were taken and subjected for successive solvent extraction. The extraction was carried out with solvents of increasing polarities: petroleum ether, chloroform, ethanol and water. The extracts were concentrated at reduced pressure through rotavapour. The extracts were preserved aseptically in brown bottle at 4°C for further studies (Ashwani et al., 2014).

Phytochemical Screening of Extracts

Qualitative phytochemical analysis of different parts of M. concanensis Nimmo was performed. Preliminary phytochemical analysis was carried out according to standard techniques depicted for alkaloids, flavonoids, saponins, tannins, phenols, Terpenoids, steroids, carbohydrates, protein, amino acids and oils & resins (Banu et al., 2009).

Antifungal Activity Assay

Antifungal activity of extracts was determined by disc diffusion method on Sabouraud Dextrose agar medium. Discs was made in Sabouraud dextrose agar plates with the assist of sterile cork borer (5mm) and inoculum containing fungi was scattered on the solid plates with the help of a sterile swab moistened with fungi suspensions. Then 50µl each of aqueous and all organic solvent extracts was located in the discs made in inoculated plates. The plates were incubated for five days at 25°C followed by measurement of zone of inhibition if any around the Disc in mm (Rao et al., 2013).

Antibacterial Activity Assay

Antibacterial activity of various extracts i.e., aqueous extracts and solvent extracts were determined by cup diffusion method over nutrient agar medium. Cups was made in nutrient agar plate by using sterile cork-borer (5mm) and inoculum containing bacteria was spread on the solid plates with the help of a sterile swab moistened with the bacterial suspensions. Then 50µl each of all the aqueous and solvent extracts was located in the cups in inoculated plates. The plates were incubated for 24 hours, at 37°C and zone of inhibition if any around the wells was calculated (Dugganabovana et al., 2021).

Result

Phytochemical Analysis

All the extracts were subjected to different phyto-chemical analysis. Detail of these tests are summarized in **Table 1.**

From the different tests performed it was found that carbohydrate, protein and amino acids and flavonoids were absent in all the fractions of extracts. Whereas glycosides, alkaloids, tannins and steroids were present in different fractions of extract.

TABLE 1

Qualitative phytochemical profile of extract.

Phyto chemicals	Tests	Inference			
		Petroleum ether	Chloroform	Ethanolic	Aqueous
Glycosides	Legal's test, Borntrager's test, Libermann-Burchard's test	(+ve)	(+ve)	(-ve)	(+ve)
Alkaloids	Dragendorff's, Wagner's, Mayer's, Hanger's Test	(+ve)	(+ve)	(+ve)	(+ve)
Carbohydrates	Molisch's test, Fehling's test, Benedict's Test	(-ve)	(-ve)	(-ve)	(-ve)
Steroids	Salkowski's Test, Liebermann test	(+ve)	(+ve)	(+ve)	(+ve)
Saponins	Froth's test	(-ve)	(+ve)	(+ve)	(+ve)
Flavonoids	Shinoda's test	(-ve)	(-ve)	(-ve)	(-ve)
Protein and amino acids	Million's test, Biuret and Ninhydrin test reagent	(-ve)	(-ve)	(-ve)	(-ve)
Tannins and phenolic compounds	Ferric chloride test and Gelain test	(+ve)	(+ve)	(+ve)	(+ve)

Yield Value of Different Dried Extracts

Yield values of different extracts were shown in table 2. From the results it was found that aqueous extract (20.2%) has highest yield value followed by chloroform extract (7.2%). Whereas ethanol extract has the lowest yield value i.e., 4 %.

Chloroform extract shows maximum zone of inhibition amongst all the extracts.

In this case also chloroform extract shows the highest zone of inhibition followed by petroleum ether, Ethanolic, aqueous and control.

In this case chloroform extract, petroleum ether and ethanolic extract shows similar type of results, ethanolic extract has shown the highest zone of inhibition followed by aqueous and control.

Petroleum ether extract as well as chloroform extract shows similar results as compared to other extracts.

The zone of inhibition of all the extracts against Enterobacter aerogenes is shown in the graph. It was found that chloroform had the highest and control had the lowest zone of inhibition.

TABLE 2

Extractive values of *Moringa concanensis.*

Percentage yield						
Plant name	**Part used**	**Methods of extraction**	**Petroleum ether**	**Chloroform**	**Ethanol**	**aqueous**
Moringa concanensis	Leaves	Successive solvent extraction	6.2%	7.2%	4.0%	20.2%

TABLE 3

Zone of inhibition of different extracts.

Organism	**Zone of inhibition –Mean values(in mm)**				
	Petroleum ether Extract	**Chloroform Extract**	**Ethanolic Extract**	**Aqueous Extract**	**Control Extract**
A. Flavus	14.16667	16.15	13.88333	14.53333	4.133333
A. Niger	18.7	19.43333	15.53333	14.7	5.466667
Helminthosporium	16.45	16.583	17.26667	13.38333	6.733337
Bacillus subtilis	17.25	17.7	14.15	15.71667	1.0111
Enterobacter aerogenes	20.45	22.18333	17.31667	19.66667	0.9233
Bacillus cereus	18.98333	20.13333	22.21667	17.23333	1.1337

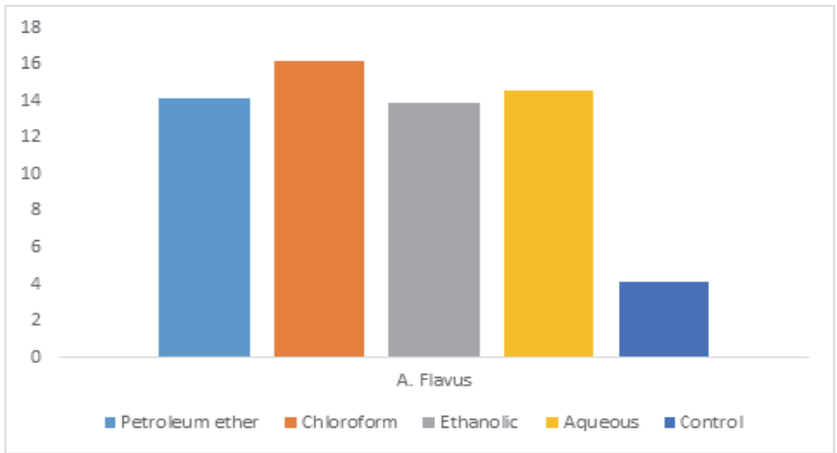

Fig. 1. *Zone of inhibition of A. flavus in all fragments of extract.*

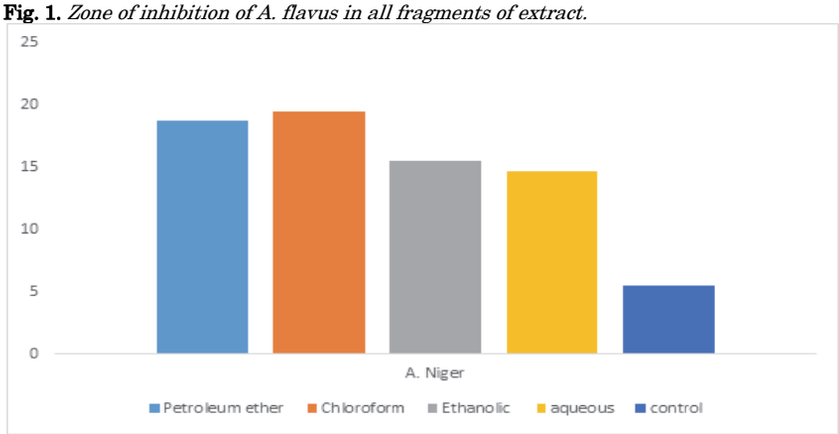

Fig. 2. *Zone of inhibition of A. niger in all fragments of extract*

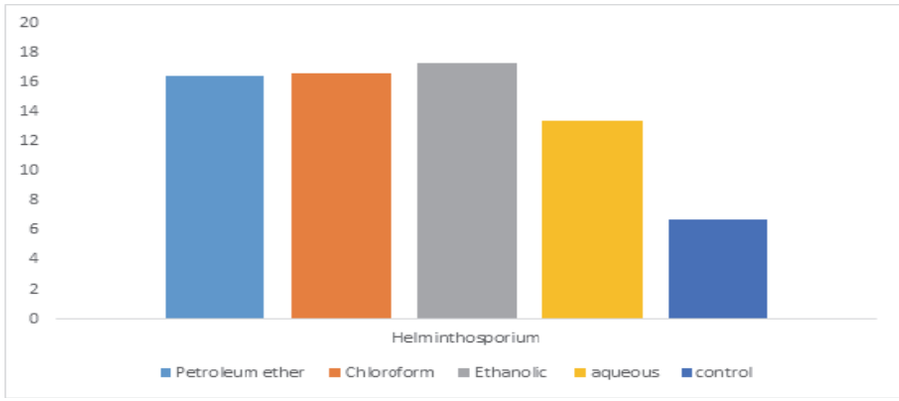

Fig. 3. *Zone of inhibition of Helminthosporium in all fragments of extract.*

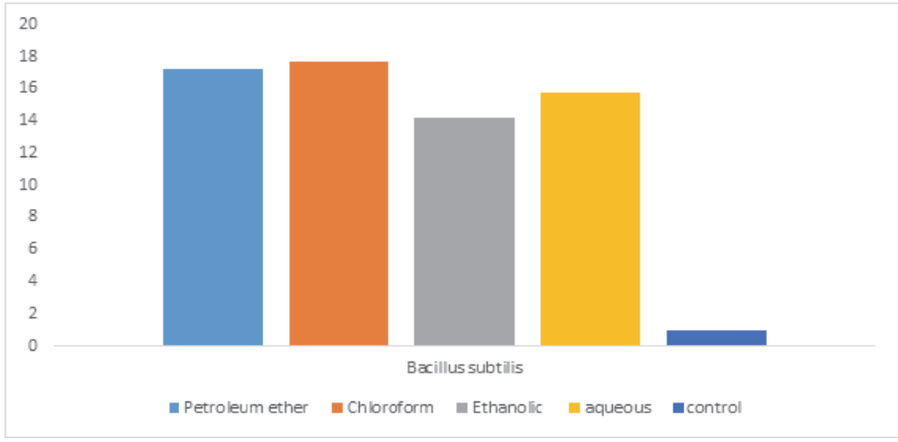

Fig. 4. *Zone of inhibition of Bacillus subtilis in all fragments of extract.*

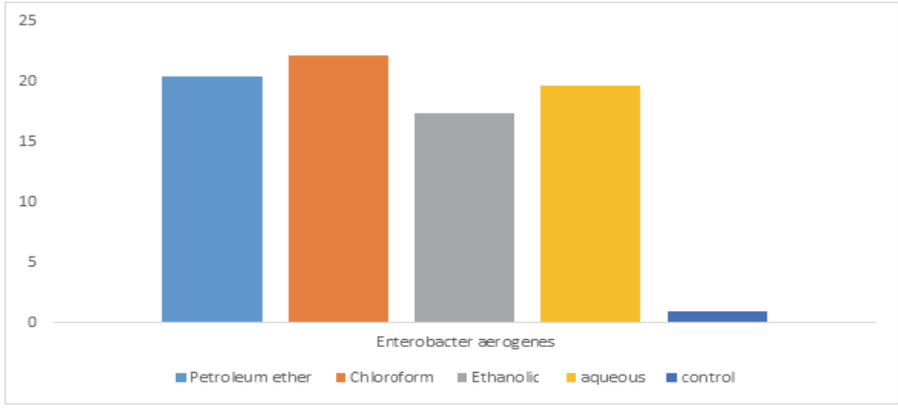

Fig. 5. *Zone of inhibition of Enterobacter aerogenes in all fragments of extract.*

The pattern of zone of inhibition of all the extracts was ethanol being the highest, followed by chloroform, petroleum ether, aqueous, control.

Discussion

The results of the Antibacterial activity is encouraging, where chloroform extract shows highest zone of inhibition amongst all the extracts, while petroleum ether, ethanol and aqueous extract showed significant activity against the control solution.

With respect to individual strains, Bacillus subtilis was inhibited most by chloroform extract, petroleum ether had comparable inhibition with marginal difference, Enterobacter aerogenes was inhibited most by chloroform extract, closely followed by petroleum ether extract and Bacillus cereus was most inhibited by ethanolic extract followed by chloroform and petroleum ether extract. Aqueous extract has the highest extractive value but shows the least inhibition among the four extracts.

Antifungal activity was performed with three species available, namely, Aspergillus flavus, Aspergillus niger and Helminthosporium.

Aspergillus flavus was inhibited by chloroform extract the most, closely followed by aqueous extract, all four extracts showed significant activity compared to control.

Aspergillus Niger was inhibited by chloroform extract the most, closely followed by petroleum ether extract, all four extracts showed significant activity compared to control.

Helminthosporium was inhibited by ethanol extract the most, closely followed by chloroform extract, all four extracts showed significant activity compared to control.

The results agreed with the traditional claim that the leaf of *M. concanensis* possess antibacterial and antifungal activity.

Conclusions

The results indicate that *Moringa concanensis* possesses significant antibacterial and antifungal activity and phytoconstituents can be further isolated to study their individual effects and determine lead compound. As the duration of this work was short, a long duration research work can be carried to find the various active phytoconstituents and their minimum inhibitory concentration. *Moringa concanensis* is an effective antibacterial and antifungal drug and needs further investigation.

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