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Phytochemical Screening, Qualitative Analysis and Isolation of Endophytes from Selected Medicinal Plants

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Abstract

The medicinal plant *Azadirachta indica* and *Moringa oleifera* have great nutritional values and are being studied here. Even every part of these plants like leaves, stem bark, roots, seed, pods have its own nutritional value and positive impact on human health. Both of these medicinal plants bears important properties like anti-bacterial, antitumor, anti-cancer, antiviral, anti-inflammatory, immunity booster.

Analysis of bioactive compound of these medicinal plants can provide a helping hand to pharmacy for treatment of such kind of diseases. These medicinal plants possess Phytoconstituents like steroids, alkaloid, saponins, tannins, and flavonoid. Endophytes (both Bacteria and fungi) are also found on these medicinal plants. Phytochemical screening of extract by using standard method like salkowaski test for steroid and that for saponin foam test showed result that of *Azadirachta indica* (leaves) in both the solution (n- hexane and distil water) showed absence of steroid and saponins. During analysis of extract of *Moringa oleifera* (leaves) with n- hexane showed absence of steroid and saponins but in the case of distil water there is presence of steroid and saponins. On the other hand the leaves of both plants contain bacterial and fungal endophytes. Leaves of *Moringa oleifera*and *Azadirachta indica* contains some bacterial and fungal endophytes. The results with *Azadirachta indica* leaves showed that the bacterial colonies (on TSA media) appeared at 10^{-2} and 10^{-3} and the fungal endophytes (on SCA media) were not appeared at any dilutions. Whereas the results with *Moringa oleifera* leaves showed that the bacterial colonies (on TSA media) appeared only at 10^{-2} dilution and the fungal endophytes (on SCA media) appeared at 10^{-3} and 10^{-4} dilutions

Key words: Medicinal plants, *Azadirachta indica*, *Moringaoleifera*, phytochemical screening, Qualitative analysis, endophytes (Bacteria and Fungi).

Introduction

India is regarded as the botanical garden and largest producer of medicinal herbs in the world (16).Medicinal plants play a significant role in human health (38). Medicinal plants are found to contain rich sources of nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates, and chemical entities of synthetic drugs (14). From the ancient time 1600-4500 BC, “Rig-Veda” depicts the use and application of various medicinal plants in remedial diseases. In Ayurveda the specific properties of drugs and various aspects of medicinal plants are found to contribute great impact to the science (17).

Herbal medicines are used to treat

almost all diseases from long back history and important to explore wide scope applications of medicinal plants (30). Infact application of medicinal extracts from the plants is still unexplored. The treatment methodologies and health care aspects of essential natural products were studied (30). Traditional plant based medicines for primary healthcare need is followed in underdeveloped countries of about 80% of world's population (WHO). The use of herbal medicines is cost-effective, robust and causes no side-effects in comparison to synthetic drug formulations to treat various infectious diseases. In India, 9500 herbal medicinal plants and also 8000 higher plants have been used in the medicinal purpose (35).

The natural ingredients found in phytoextracts are current need for the development of method for establishing quality control parameters for Ayurvedic formulation owing to variability and complexity of chemical constituents present in herbal plant based drugs.

Herbal medicine was the lifesaving drug as compared to modern medicine. Amid 4, 00,000 plant species only 6% of the plants are studied for their biological activity and only few have been phytochemically investigated. This data shows that the inspection is needed for many medicinal plants for its activity and pharmacological properties. The development of herbal medicine was done by the primary screening of the compound in the plant extract (30). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (25). Plants are the crucial storehouse of many chemical metabolites. Basically they are divided into two categories primary and secondary metabolites. Primary metabolites involved directly in growth and metabolism of the plants and secondary metabolites involved in their defense mechanism and not involved in metabolic activity of plants. Secondary metabolites include alkaloid, terpenoid, flavonoid, saponin, phenol, glycoside, tannin, steroid etc. studies on secondary metabolites revealed that these compounds have antioxidant, antibacterial, anticancer anti-inflammatory, antitumor, antiviral, and many other actives (18).

Secondary metabolites are not necessary part of plant's life but contribute to the species fitness for survival. They protect plants against both biotic and a biotic stress, biotic stress due to bacteria, fungi, nematodes, insects and grazing by animals and a biotic stress due to higher temperature and moisture, shading, injury or presence of heavy metals. Secondary metabolites used by human as especially chemical such as drugs, flavors, dyes, fragrance, and insecticides because of a great economic value.

Secondary metabolites mainly divided in three major groups they are polyphenol compounds, including flavonoids and phenols, terpenoids and alkaloids. Flavonoids are found in all parts of higher plants including leaves, roots, flowers, fruits, wood, skin, pollen and seeds of varying levels (40).

Through the medicinal plants secondary metabolites play an important role in healthcare system. Secondary metabolites are identified by the GC-MS analysis (3).

In the last few years, Gas chromatography Mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolites profiling in both plant and non-plant species. Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. However, few reports are available with respect to the pharmacological properties of the plant (16). The aim of this research is to identify phytochemical in two medicinal plants *Azadirachta indica* and *Moringa oleifera* by GC-MS analysis.

The chemical constituents incorporate various biologically energetic compounds that can be extracted from neem like alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids etc. (7).

Moringa oleifera (sahajan) is a drought tolerant, fast-growing, multi-purpose and one of the most useful tree due to medicinal and nutritional properties in world, therefore described as a “Miracle tree”. *Moringa* plant commonly available in India, its wild source rarely but cultivated in everywhere in India. *Moringa* has long been used in herbal medicine by Africans and Indians, frequently referred as a panacea and can be used to cure more than 300 diseases*. Moringa oleifera* is the most hopeful tree because of its nutritional benefits, medicinal properties, environmental conservation and consumption and is the perennial multipurpose. Reputedly it is known as “cabbage tree”, “drumstick tree”, “horseradish tree”, “benzoil tree”, “miracle tree” and “mothers best friend tree*”. Moringa* leaves are a storehouse of nutrients, rich in mineral, vitamin (vitamin A (beta-carotene), vitamin B-choline, vitamin B1-thiamine, riboflavin, nicotinic acid and ascorbic acid), fiber, fat, protein, amino acid and folic acid. The plant is reported to be used in phytomedicine as antioxidant, antimicrobial (antifungal, antibacterial), anti-inflammatory, antipyretic, antiulcer, anti-diabetic, anti-tumor properties as well as its wound healing properties has been demonstrated (19).

Moringa is quite potential but still less explored. *Moringa* has high tannin, phenol and triterpenoid content, flavonoids, saponins and alkaloids which are very likely to be developed as medicinal plant (23).

Ironically, in recent years, microorganisms associated with plants rather than plants they have proved to offer material and products with high therapeutic potential (11). Word endophyte means “in the plant”; endophytes are microorganisms (mostly fungi and bacteria) that inhibit plant hosts for cell or part of their life cycle. Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissue intracellularly or intracellularly without causing any apparent symptoms of diseases (34). Endophytes can colonize in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruits, buds, seeds. Endophytes are ubiquitous in all plant species and produce an array of secondary metabolites or accumulate bioactive secondary metabolites that may assit plant defense and leads in drug development (21).The functional metabolites produce by endophytes include alkaloids, terpenoids, steroids, quinones, flavonoids, phenols and phenolic acids.

Elzein M. Fahal *et al,* stated about medicinal properties and nutritional value of diffrents parts(leaves, seeds, pods, roots and bark.) of plant *Moringa oleifera* (Lam.) *Moringa oleifera* (Lam.) have different bioactive compounds such as alkaloids, flavonoids, saponins, sterols and tannins which may show its therapeutic effects by combination of these compounds and may be due to individual effect in aqueous, ethanol, methanol and chloroform extracts of this plant showed existing of these five important phytol-constituents. Moringa pods crude extract also passed through Quantitative analysis for the same metabolites in it (10).

The major objective of this study is related with extraction of secondary metabolites because of their medicinal valuesand isolation of endophytes from *Azadirachta indicia* and *Moringa oleifera.*

Materials and Methods

(A) phytochemical screening

Collection of plant samples

The medicinal plants used for the experiment were leaves of *Azadirachta indicia* (neem) and *Moringa oleifera* (sahajan), (17). These plants were cultivated and harvested from Churu Rajasthan, and then fresh leaves of these plants, shade dried in open air without

sunlight for 10 days. Dry leaves were chopped until smooth then pulverized to powder using mechanical grinder until soft (15). Powder was stored in air-tight bottles. The powder was used for extraction procedure and photochemical analysis (18).

Plant sample extraction

This experiment requires 10 gm of *Azadirachta indica* and *Moringa oleifera* and two beakers of n-hexane with equal volume of 250ml in each and also of distill water with same parameter. All this process followed by adding 5gm *of Azadirachta indica* leaves powder in one beaker of n-hexane (250ml) and another 5gm in distill water(250ml). Then same things were done with *Moringa oleifera* leaves powder (12). The solutions were heated at their boiling point 68°C (n-hexane) and 100°C (water) in the water bath until the solutions become colorless (12). The extracts were collected and filtrated by using Whattman filter no. 1. These extracts were used for the phytochemical analysis and GC-MS analysis to find the bioactive components (30). Finally, the extracts stored in clean, dried beakers at 4°C (3).

Fig. 1: *Moringa oleifera* plant and leaves

Fig. 2: *Azadirachta indica* plant and leaves

(B) isolation of endophytes

Collection of plant samples

Fresh, healthy, undamaged mature green leaves and senescent leaves were collected by selected medicinal plants *Azadirachtaindica*and *Moringa oleifera*, from Churu region, Rajasthan (28). The collected leaves were brought to the laboratory and instantly processed for isolation of endophytes.

Isolation of bacterial and fungal endophytes

Endophytes can be easily isolated on any microbial or plant growth such as agar, potato dextrose agar and any nitrogen or carbon containing media. The most detect method used to detect and enumerate endophytes involves isolation from surface sterilized host plant tissue (11).

The leaves were weighed up to one gram on a weighing balance. The weighed samples were soaked in distill water and drained the samples on sterile filter paper were then surface sterilized by dipping in 70% ethanol (EtOH) for 1minutes, then treated with 4% sodium hypochlorite (NaOCl) for 5 minutes, then treated with 70% ethanol for 30 seconds followed by rinsing five times in sterilized distill water. The surface sterilized samples were blot-dried using sterile filter paper under aseptic conditions. These surface sterilized leaves samples were then macerated separately in 1ml of phosphate buffer (pH 7.2) with a sterile pestle and mortar. Tissue extract were then prepared for tenfold serial dilution. About 1ml of each macerated sample (*A.indica* and *M. oleifera* leaves sample) was serially diluted up to 10^{-4} dilutions. About 0.1ml (100 µl) of aliquot from 10^{-2} to 10^{-4}dilutionsof the respective samples were then poured in their respective autoclaved Petri plates containing TSA (Trypticase soy agar) media and SCA (Sabouraud chloramphenicol agar) media, and then spread with sterile spreader for isolation of Bacterial endophytes and Fungal endophytes. For isolation of bacterial endophytes, the plates

were incubated at 37°C for 72 to 96 hours. For isolation of fungal endophytes, the plates were incubated at 28°C for 14 days.

Results and Disscusion

Results for phytochemical screening

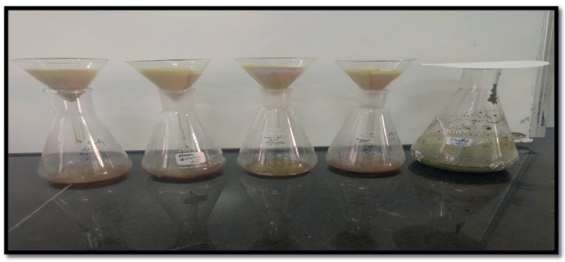

Fig. 3: Plant sample extraction

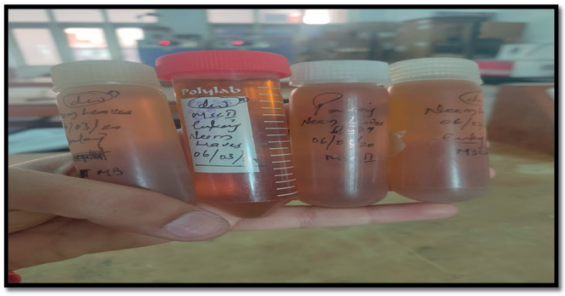

Fig. 4: Plant sample extraction

(1) The phytochemical tests (Qualitative Analysis) were done by *Azadirachta indica and Moringa oleifera* plant extracts with two different solvents n-hexane and distils water. The results were attended in following tables.Phytochemical screening:

(2) Screening of the above two selected medicinal plants A. indica, M. oleifera for phytochemical constituents were carried out using standard method.

Table 1: Phytoconstituent analysis

Phytoconstituents	Tests	Observation
Steroids (Salkowski test)	2ml extract +2ml $CHCl_3$+ 2ml H_2SO_4(conc.)	Red color
Saponins (Foam test)	5ml extract+ 5ml H_20+heat	Froth appears

Table 2: Phytochemical screening of leaves of *Azadirachta indica*

SN.	PLANT CONSTITUENTS	n-HEXANE EXTRACT	DISTILL WATER EXTRACT
1.	Steroids	–	–
2.	Saponins	–	–

- (absent), + (present)

Table 3: Phytochemical screening of leaves of *Moringa oleifera*

SN	PLANT CONSTITUENTS	n-HEXANE EXTRACT	DISTILL WATER EXTRACT
1.	steroids	_	++
2.	saponins	_	++

- (absent) + (present)

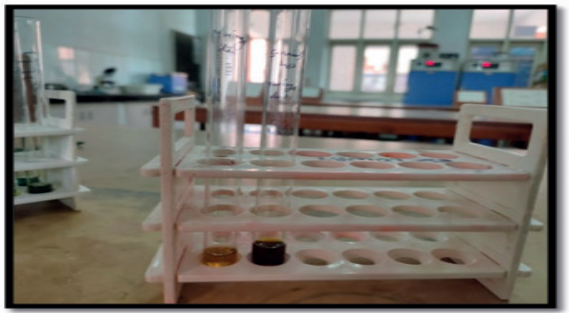

Fig.5: *Moringa olifera* Distil water extractSteroid testIndicating test - positve

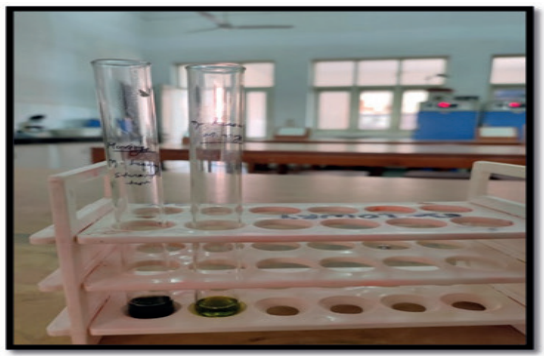

Fig. 6*: Moringa olifera* n-hexane extract steroid test Indicating test -negative

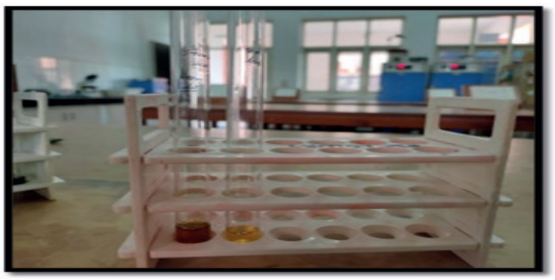

Fig. 7: *Azadirachta indica*Distil water extract Steroid testIndicating test -negative

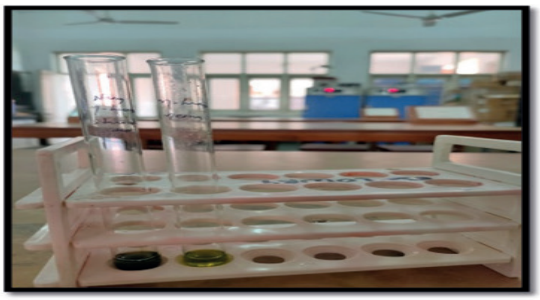

Fig. 8: *Azadirachta indica* n-hexane extract Steroid test Indicating test -negative no red color

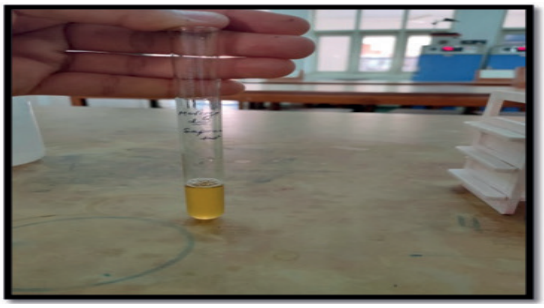

Fig-9. *Moringa olifera* distilled water extract saponin test Indication test- froth formation

Fig-10: *Moringa oliafera Hexane extrecrt Saponin test* Indicating *test- negative*

Fig-11: *Azadirachta indica*n-hexne saponin test Indicating test- negative

Isolation of Endophytes

AzadirachtaindicaFungal endophytes (SCA medium)

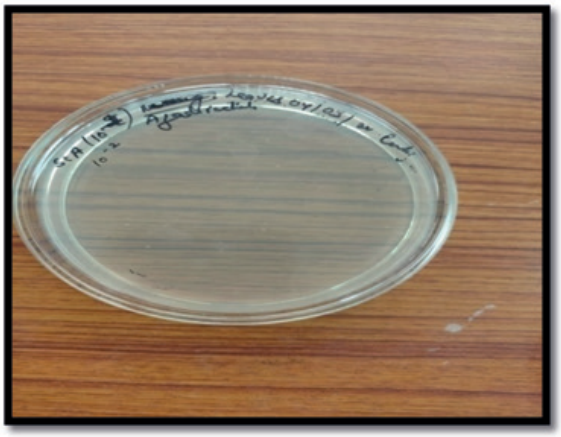

Fig. 12. 10^{-2} dilution- no growth apperared

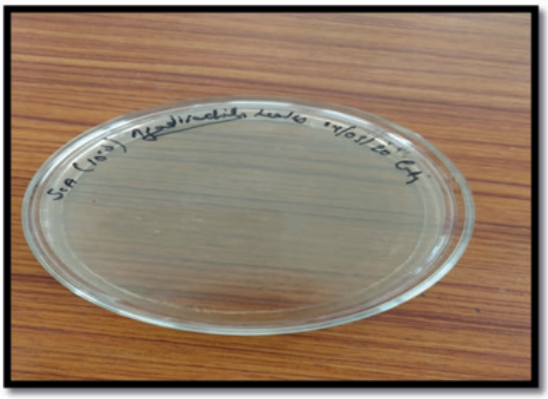

Fig.13.10^{-3}dilution- no growth apperared

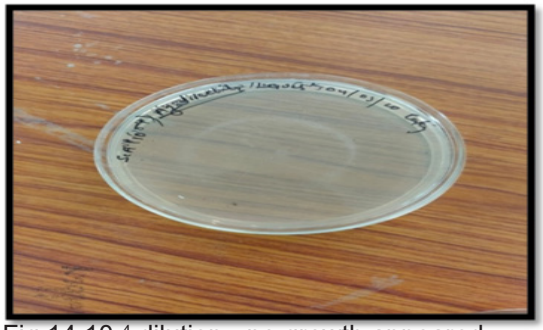

Fig.14.10^{-4} dilution - no growth appeared Azadirachta indica bacteial endophyte (TSA medium)

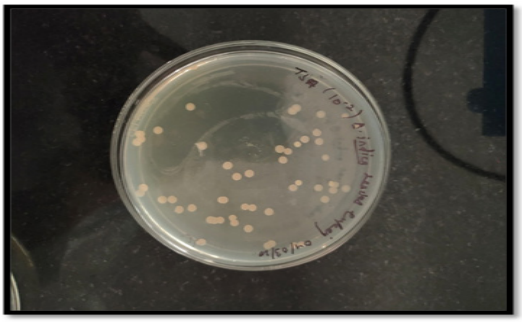

Fig.15.10^{-2} dilution colonies of bacterial endophyte

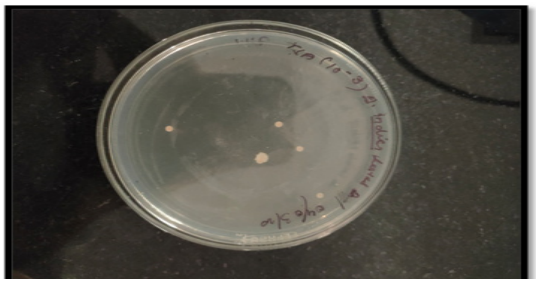

Fig. 16. 10^{-3} dilution colonies of bacterial endophyte

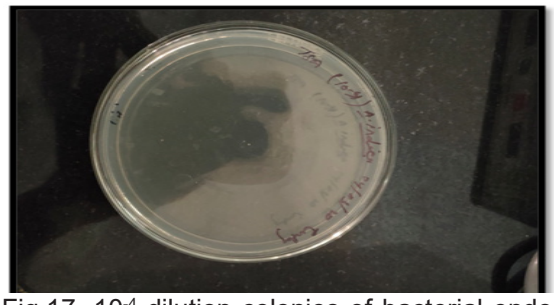

Fig.17. 10^{-4} dilution colonies of bacterial endophyte

Moringa oleifera (Fungal endophytes) (SCA medium)

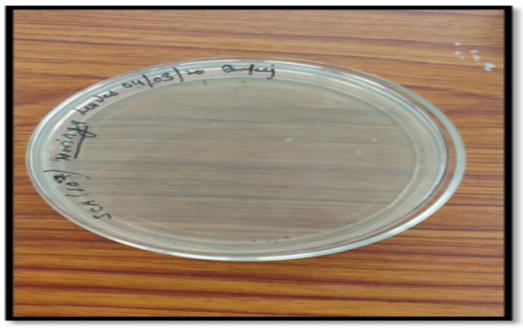

Fig.18. 10^{-2} dilution –no growth appeared

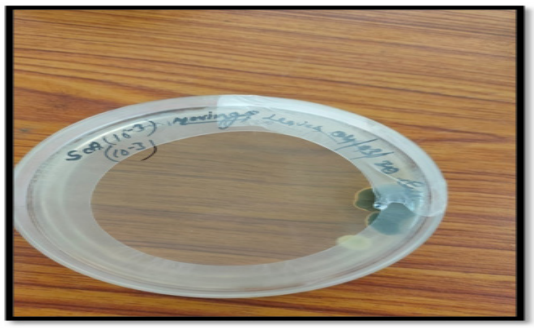

Fig.19. 10^{-3} dilution colonies of fungal endophytes

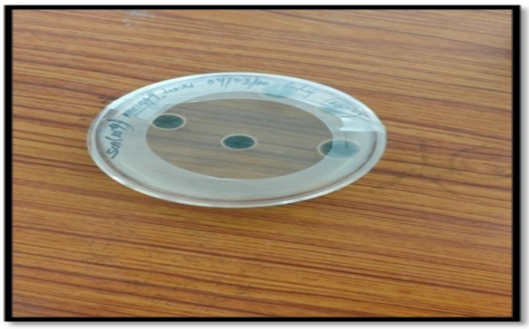

Fig. 20. 10^{-4}dilution- colonies of fungal endophytes

Moringa oleifera (Bacterial endophytes) (TSA medium)

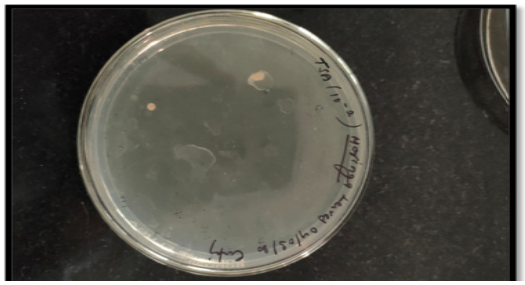

Fig. 21. 10^{-2} dilution- colonies of bacterial endophytes

Isolation of endophytes from selected medicinal plants

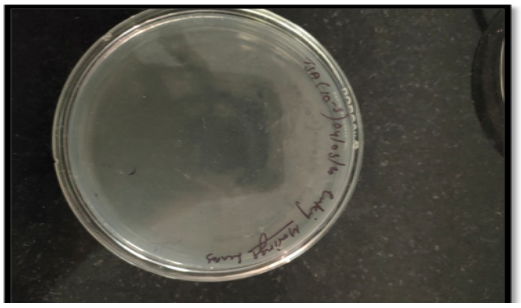

Fig. 22.10-^{3} dilution- no growth

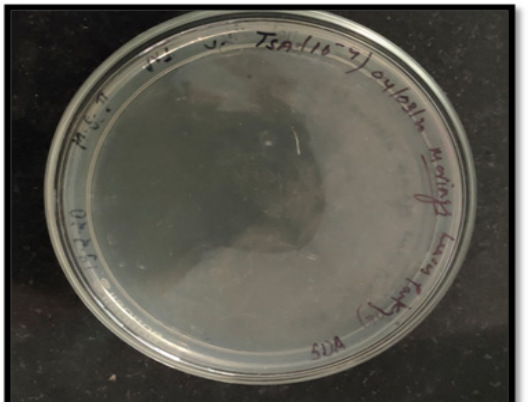

Fig. 23. 10-^{4} dilution- no growth

Discussion

Traditional folk medicines from plants have always directed scientists to search for new medications in order to maintain and promote healthy life for human and animals. Herbal medicine contain some organic substances which provide definite physiological action on the human body as well as their physiological activities due to the presence of secondary metabolites include tannins, alkaloids, flavanoids, glycosides, steroids, glycosides (3,9). Basically phytochemical screening or qualitative analysis is used to reveal the chemical constituents or the secondary metabolites of the plant extracts or tissue in different plant parts (10). Phytochemical process was carried out among the two medicinal plant extracted to the two different solvent are processed to determine the phytochemical constituents. The name of the solvent n-hexane and distill water (13). In the present study, determine the preliminary phytochemical analysis of n-Hexane and distill water extracts of medicinal plants: *Moringa oleifera*and Azadirachta indica. The study of the bioactive constituents (secondary metabolites) of the medicinal plants has acquired a lot of significance all over the world. The plants samples were collected, shade dried and powdered were subjected to phytochemical screening. The dried powder leaves of Moringa oleifera and *Azadirachta indica* subjected to extraction with n-hexane and distill water (33). After maceration in n-hexane and distill water the as-obtained extracts were then examined through several procedure. The first step is preliminary phytochemical assay to qualitatively analyze the Phytoconstituents in the extracts. The second step is Performing GC-MS technique to analyze and predicted the structure of bioactive volatile compounds. Many workers (31) have reported phytochemical screening of various plants. The presence of wide range of phytochemical constituents indicates that the plants could be used in a multitude of ways, which may be beneficiary to the population (27). Qualitative analysis of phytochemical constituents was area that is more interesting and important application of biomedical in pharmaceutical industries. This phytochemical analysis was very useful finding chemical compound in the plant material that lead to their quantitative estimation and locating the pharmacy field, (20, 22, and 29). The present work the phytochemicals were detected by color tests. The investigation revealed that the Steroids and saponins were absent in n-hexane and distill water extracts of *Azadirachta indica* leaves (Table No.2) where only distill water extracts of *Moringa oleifera* leaves contains steroids and saponins, steroids and saponona were absent in n-hexane extracts of Moringa oleifera leaves (Table No.3). Endophytes are ubiquitous in all plant species and play an important role in their host plant defense and tolerance to stress condition. Endophytes produce abundant metabolites that helpful in plants tough conditions like that defense and stress condition. Endophytes (both bacteria and

fungi) produce toxins, pharmaceuticals, enzymes and growth factors, due to these characteristics endophytic microorganisms have received attention and guided to scientists for research. Numerous studies on endophytic micro biota revealed that the important information about plant and environmental interaction. Numerous studies reported the isolation of large number of endophytes from medicinal plants.In this work isolated the endophytes from leaves of *Moringa oleifera* and Azadirachta indica.For isolation, collected the plants leaves sample and disinfected by surface sterilization method and macerated with phosphate buffer (pH 7.2). After maceration, extracts were subjected to serial dilution and grow on TSA media for bacterial endophytes and SCA media for fungal isolation. Results were shown in previcious figures of bacterial and fungal endophytes of both medicinal plants. The results proved that the leaves of *Moringa oleifera* and contain some bacterial and fungal endophytes. The results with *Azadirachta indica* leaves showed that the bacterial colonies (on TSA media) appeared at 10^{-2} and $10^{-3}$and the fungal endophytes (on SCA media) were notappeared at any dilutions.

The results with Moringa oleifera leaves showed that the bacterial colonies (on TSA media) appeared only at 10^{-2} dilution and the fungal endophytes (on SCA media) appeared at 10^{-3} and 10^{-4} dilutions. From my research finding it is vary cleared that the phytoextract from the plant *Moringa oleifera* and *Azadirachta indica* was closely compared with finding of Sahal *et al* experiment.

Conclusion

In this study can conclude that the selected distill water leaf extracts of Moringa oleifera contain steroids and saponins but the n-hexane leaf extract does not contain the steroids and saponins in other hand the both n-hexane and distill water leaf extracts of Azadirachta indica does not contain any type of steroids and saponins.

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Conflict of Interest:

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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