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PRELIMINARY PHYTOCHEMICAL SCREENING OF SUCCESSIVE REEXTRACTION OF *Moringa oleifera* LEAVES.

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Abstract*:* The succeeding reextraction of plant *Moringa oleifera* leaves of four different solvents using n-hexane, ethyl acetate, methanol and water for the preliminary phytochemical screening and is the focus of this study. Screening of phytochemicals with the help of a dried powdered sample of *M. oleifera* leaves extract by successive reextraction of solvent n-Hexane, ethyl acetate, methanol and water extract to be tested for various primary and secondary metabolites. The result shows the presence of phytoconstituents (anthocyanins, flavonoids, tannins, saponins, alkaloids, phytosteroids, phenols, protein, amino acids, and carbohydrates of selective solubility in their respective solvents of varying polarity are used consecutively. The presence of phytoconstituents of testing results in the successive extract is suggesting that importance of solvent and their solubility factors of *M. oleifera* leave extract, which is a nutritionally rich dietary supplement of diver's bioactive compounds these information data of active principle of medicinal perspective is help isolates and characterizing of pharmacological importance of phytomedicinal value.

Keywords: - *Moringa oleifera*, phytoconstituents, leaves, successive, extraction.

Introduction

Nowadays Era has come for herbal medicine to treat various aliments directly or indirectly. The plants contained very rich amounts of macro and micronutrients. Various medicinal plants have been used for dietary supplements as well as medicinal perspective. *Moringa* plants are one of them, it has commonly known as munga or drumstick also refers as a miracle tree (Lowell and Fuglie 1999). It is a tropical deciduous perennial dicotyledonous tree, belonging to the family moringaceae, their size is about 10-100m long. Their leaves are green and bi-pinnate mostly tripinnate compound. The sub-Himalayan mountain of northern India is its native region its cultivated tropical and subtropical region of the world for various purposes of variety. It is a rich amount of vitamins, minerals, and proteins. All plant parts of this tree are very useful for nutritive as well as medicinally for the management of various therapeutic purposes (Mishra et al., 2011, Fahey 2005). In addition, the *Moringa oleifera* leaves contain antioxidants, antimicrobial, anti-carcinogenic, antibiotic, and antisickling activity for various pharmacological purposes.

The extensive research work proved that plant is used as phytomedicine for the management of various ailments. This plant contains various bioactive compounds such as alkaloids, saponin, tannin, flavonoids, terpenoids, and anthocyanins. The plant *Moringa oleifera* leaves contain to above of their phytochemicals. The present study, aimed to preliminary phytochemical screening of successive reextract of the plant *Moringa oleifera* leaves in successive manners of solvents n-Hexane, ethyl acetate, methanol and water.

Taxonomic Classification

Kingdom- Plantae

Division- Magnoliophyta

Class- Magnoliopsida

Order- Capparales

Family- Moringaceae

Genus- *Moringa*

Species- *oleifera*

Fig. *Moringa oleifera* leaves (drumstick)

Materials and Methods

Collection of Sample - In the present study used plant parts collected from Bhakhara Nagar in the Dhamtari district.

After collection, sample parts were washing with running tap water followed by distilled water to remove dust and another harmful particle.

Drying and Grinding - After washing the plant sample were dried in the shade for 20 days and then ground into fine powdered form then store until further use.

Hot Extraction - The dried powdered sample of M. oleiferea leaves was weighed 50g by the weighing machine, and after that put into a thimble to process successive reextraction. The hot extraction was done by each 250ml of four different solvents respectively to increase their polarity n-Hexane, Ethyl Acetate, Methanol and water with the help of Soxhlet Apparatus. After that, each extract obtained following successive reextraction was filtered using Whatman Filter paper No. 1 and then dried threw a water bath as semisolid mass and yield of each extract thus obtained was recorded and stored in a refrigerator at 4o C till further use (Roopalatha 2013).

Phytochemical Screening - Each successive extract obtained using their respective solvent prepare a 1% concentration stock solution. It is used for phytochemical analysis to identify the phytoconstituents with the help of standard procedures previously described by (Harborne 1998, Treas and Evans 1989, Sofowora 1993, and kandalwal 2005).

Screening for Preliminary Phytochemicals

Test for Primary metabolites

Carbohydrates -

Benedict's test - 1ml extract in a test tube then 1ml of benedicts reagents was added, the mixture is heated in a boiling water bath for 2 minutes greenish solution appeared showing the presence of carbohydrates.

Protein -

Xanthoproteic test - 1ml extract were treated with a few drops of concentrated nitric acids, formation of yellow colour indicates the presence of proteins.

Amino Acids -

Ninhydrin test - 1ml extract to add a few drops of ninhydrin reagent with purple colour appearance to indicate the presence of amino acids.

1ml extract adds a few drops of nitric acid along the side of the test tube yellow colour appearance to indicate the presence of amino acids.

Test for Secondary Metabolites -

Anthocyanins -

1 ml extract were taken to add 2N HCl and it was followed by the addition of ammonia the conversion of pink-red turns blue - violet indicating the presence of Anthocyanins.

Alkaloids -

Wagner's reagent test - 1ml extract were added to a few drops of Wagner's reagent along the sides of the test tube, a reddish-brown precipitate appearance indicates the presence of alkaloids.

Mayer's reagent test - 1ml extract were added 1ml of Mayer's reagent along the side of the test tube turbidity shows the presence of alkaloids.

Flavonoids -

Alkaline Reagent test - 1ml extract was intermixing with 10% sodium hydroxide solution, intense yellow colour shows the presence of flavonoids.

Zn dust test - 1ml extract was treated with Zn dust and conc. HCl added formed to red colour indicates the presence of flavonoids.

Phenols -

Ferric Chloride test - 1ml extract treated with few drops of alcoholic ferric chloride, formation of bluish-black colouration indicates the presence of Phenols.

Phytosteroids -

Liebermann-Burchards test - Extracts were dissolved in 1ml of acetic anhydride to add a few drops of conc. Sulphuric acid along the side of the test tube an array of colour changes shows the presence of phytosteroids.

Saponins -

Foam test - 2ml of the extract was mixed with 5ml of distilled water and shaken vigorously, the formation of froth shows the presence of Saponins.

Tannins -

Ferric Chloride test - 1ml extract with a few drops of 5% ferric chloride solution was added, formation of greenish colour indicated the presence of Tannins.

Lead Acetate test - 1ml of extract to add a few drops of 1% lead acetate solution, yellowish precipitate shows positive intent of Tannins.

Results and Discussion -

The phytoconstituents in plant extracts are active principles of various drug investigations from the pharmacological point of view to find various secondary metabolites.

In these studies, phytochemical screening of plant Moringa oleifera leaves of successive reextraction of different solvents (n-Hexane, Ethyle Acetate, Methanol and Water) highest yield is recorded in the case of methanol followed by water ethyl acetate and n-Hexane (table 1).

The plant Moringa oleifera leaves successive reextracts showing the presence of various phytoconstituents, of preliminary phytochemicals screening in successive reextraction (table 2).

Table 1: The yield and colour of the extracts of Moringa oleifera leave obtained following extraction with n-Hexane, ethyl acetate, methanol and water in succession.

Solvent used	Sample(g)	Boiling Points(^{0}C)	Total hrs of Extraction	Yield(g)	Yield/100g	Colour of extract
n-Hexane	50g	68	4.5hrs	1.46	2.79	Yellowish green
Ethyl Acetate	48g	77	5hrs	2.43	4.41	Light green
Methanol	45g	65	8hrs	10.89	19.92	Dark green
Aqueous	33g	100	7hrs	5.81	10.69	Dark green
Residue left	25g					

Table 2:- Phytoconstituents screening in the successive extracts of leaves *Moringa oleifera* obtained from four different solvents (n-Hexane, Ethyl Acetate, Methanol and water).

S. No.	Phytoconstituents	n-Hexane	Ethyl Acetate	Methanol	Water
1.	Carbohydrates	-	-	+	+
2.	Protein	-	-	+	-
3.	Amino Acids	-	-	+	-
4.	Anthocyanins	-	-	+	+

5.	Alkaloids	+	+	+	+
6.	Flavonoids	+	+	+	+
7.	Phenols	-	+	+	+
8.	phytosteroids	+	+	+	+
9.	Saponins	-	-	+	+
10.	Tannins	-	-	+	+

The results showed Phytoconstituents were mostly present in methanolic extract followed by aqueous and the least amount of n-hexane extracts. The result of present screening on the *M. Oleifera* leaves successive reextract are as follows: Primary metabolites - carbohydrates protein and amino acids were present in the methanolic extract. Secondary metabolites- Anthocyanin were present in methanol and water extract of *M. Oleifera* leaves, whereas n-Hexane and ethyl acetate shows negative results. Alkaloids, Flavonoids and phytosteroids present in all extracts of *M. Oleifera* leaves; Phenols are only absent in n-Hexane, whereas ethyl acetate, methanol and water show positive results; Saponin and tannin are present in methanol and water extract, whereas absent in n-Hexane and ethyl acetate extract of *M. Oleifera* leaves. The alcoholic extract of M. Oleifera leaves has been anthelmintic activity (Hung et al., 2007), the effect of leaves extract of *M. Oleifera* leaves on sickle cell haemoglobin (Nwaoguikpe et al., 2015), successive reextracts of *M. Oleifera* leaves presence various phytoconstituents (Roopalatha and Vijiya 2013).

The earlier researcher reported on preliminary phytochemicals screening using single solvents at a time for the presence of phytoconstituents on the *M. Oleifera* leaves extract. In this study screening using n-Hexane, ethyl acetate, methanol and water extraction in successive reextracts manner to obtain the phytoconstituents by their varying polarities properties to use consecutively for suggesting the importance of solvents as a pivotal factor (Koruthu et al., 2011). Further studies lead to the belief that *M. Oleifera* leaves are exceedingly nutritious supplements and as a growth fostering rich in primary metabolites (Tahiliani and Kar 2000). Advance the data excluded that the successive reextraction using solvents of varying polarities would maximize and escalate the taking advantage of the several bioactive compounds. The instant fact thus would be of help to outlying and distinguish the multiple pharmacokinetic active principles of importance supporting their varied biological activities and therapeutic values.

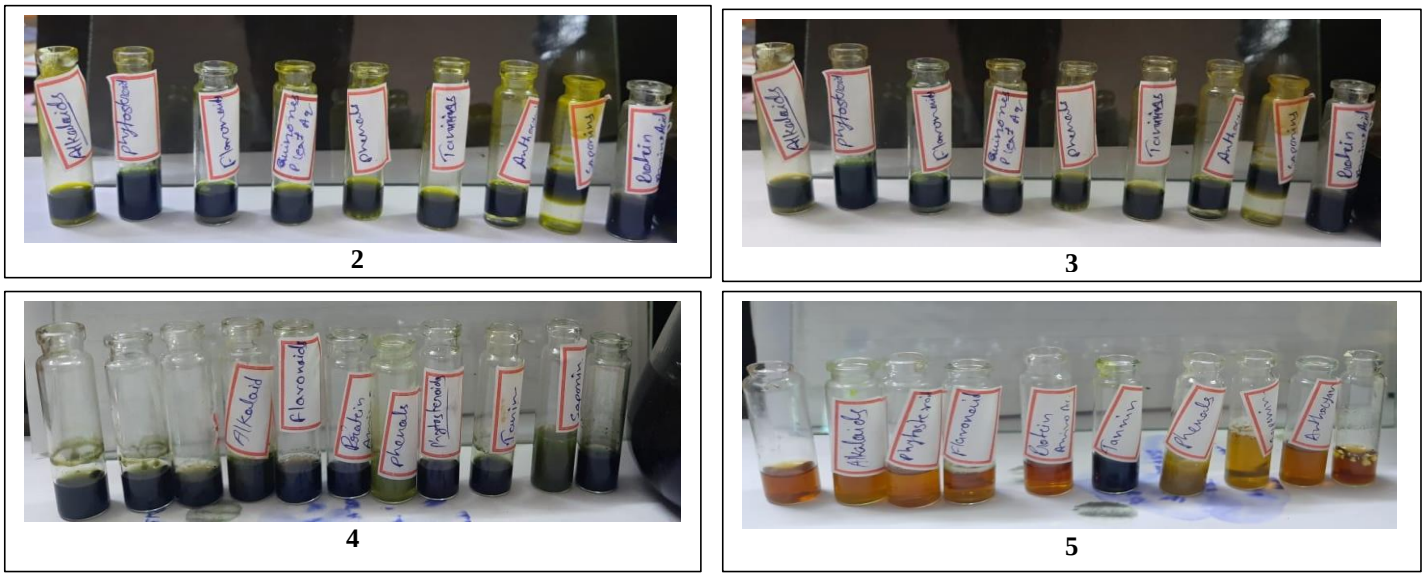

Fig. 2 to 5: Demonstration of phytochemicals screening of various extract for primary and secondary metabolites

(2) n-Hexane extract, (3) Ethyl acetate extract, (4) Methanol extract, (5) Water extract

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