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SHORT COMMUNICATION **OPEN ACCESS**

In vitro Phytochemicals analysis of selected medicinal plant *Moringa oleifera* (Lam.) by using Soxhlet extraction method

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ABSTRACT
The qualitative phytochemical studies were carried out in the solvents of Methanol, Chloroform and n-Butanol. The Methanol, Chloroform and n-Butanol leaves extract of Moringa oleifera (Lam.). showed that the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols. Total content of Alkaloid, flavonoids and phenols in the Methanol, 1.926, 0.434 and 1.641 µg/ml. respectively, in the Chloroform 1.554, 0.391 and 0.856 µg/ml. and followed by in the n-Butanol leaves extract of Moringa oleifera (Lam.). that was 2.045, 0.423 and 0.426 µg/ml.
Keyword: - *Qualitative, quantitative, Soxhlet extraction and Moringa oleifera (Lam.)*

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INTRODUCTION

Moringa oleifera (Lam) plant belonging to family Moringaceae its small deciduous tree grows up to 8 meter in height [8]. it's origin western and sub-Himalayan region and distributed thought out world, India, Pakistan Bangladesh, Nepal and rest of Asian countries not not only Asian countries but this plant found Africa and European countries [11]. Know days *Moringa oleifera* (Lam) cultivate throughout world for commercial purpose for vegetable in the diet form of drum stick because it is rich source of alpha carotene, beta carotene, and phytochemicas constituents such as alkaloids, flavonoids, saponins, sterols, phenols and tannins. The therapeutic effects of *Moringa oleifera* could be due to the combined actions of various bioactive components found in the plant, including trace metal ions, vitamins, alkaloids, polyphenols and other elements [3] and they collectively act on broad physiological processes including metabolism and immunity [2]. According to Morton, [12], the Wealth of India, [18], Dahot [4], leaves are useful for Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling. So, present investigation on phytochemicals analysis of *Moringa oleifera* leaves extract in methanol, n-Butanol and chloroform by using special Soxhlet extraction method.

Soxhlet extraction method was recommended for extraction of lipid first time by Ritter von Soxhlet but know days, this method used for extraction of phytoconstituent from different natural plant sources. The Soxhlet extraction is a simple and convenient method for infinitely repeated cycle of extraction with a fresh solvent until complete exhaustion of the solute in the raw material [12. During extraction with Soxhlet, the process of distillation is implicated. It consists of heating a solution up to boiling and then condensed send back to the original flask [7, 8]. Practically, a limited quantity of dry material is introduced in a thimble. This thimble is then deposited in a distillation flask fill with specific solvent. After reaching to a submersion level, a siphon absorbs the solvent in the thimble-holder and then release it back into the distillation flask. This solution contains the extracted solutes.

MATERIAL AND METHODS

Collection of plant materials

Fresh parts of medicinal plants, were collected from different regions of Marathwada region. The plant materials were taxonomically identified and the plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers with proper labelling for future use.

Preparation of Plant Part Extract

Methanol, Chloroform and n-Butanol, extract was prepared by using Soxhlet extractor. 30 gm of each plant part powder was placed in a thimble, which was placed in chamber of the Soxhlet apparatus. 300 ml solvent in the flask and the temperature was maintained at 55 0 c. for 72 hours. Then the extracts were filtered through Whatman filters paper No 1. Solvent was evaporated at 40-50 0C by using Rotary evaporator. The collected powder was weight and dissolved in Dimethyl sulfoxide (DMSO) with 10% concentration. The extracts were used for Qualitative and quantitative evaluation of phytochemical.

Qualitative analysis of *Moringa oleifera* (Lam.). plant parts

The qualitative screening test were performed for the presence of following secondary metabolites such as alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols [6] and Sofowara [17].

Quantitative analysis of *Moringa oleifera* (Lam.). plant parts

Preparation of plant extracts for quantitative determination of alkaloids 5 gm of powdered plant material was taken into 20 ml of n-butanol and vigorously stirred. The content was transferred into a reagent bottle. The slurry was kept overnight at room temperature. Then it was centrifuged at 6000 rpm for 10 min and the supernatant was made up to 50 ml with n-butanol

Estimation of total alkaloids by titrimetric methods used by Plummer. [17] and Debnath [5].

Obtained supernatant of the plant sample was used for the estimation of total alkaloids by titrimetric methods. 10 ml of the supernatant was taken into a100 ml separating funnel. 10 ml of 0.1 (N) HCl was added and shaken thoroughly for 2-3 min. This results in the solubility of alkaloids. The lower layer contains alkaloids neutralized with 0.1 (N) HCl and the upper layer contains n-butanol. 10 ml HCL portion was collected in a beaker and 2-3 drops methyl red was added to it, that turns the solution into slightly reddish colour. The contents of beaker were titrated against 0.1 (N) NaOH, till colour change changed from red to pale yellow. The neutralization point was determined. Same procedure was repeated triplicate. The total amount of alkaloids was calculated by considering the following equivalent:

1 ml 0.1N HCl = 0.0162 g alkaloid

Estimation of Total Phenolic Content

Total phenol content of *Moringa oleifera* (Lam.). was assayed by standard method. The different concentrations of 10µg, 20µg, 40µg, 60µg, 80µg, and 100µg were using an aliquot of diluted extract and added to 0.25 ml of Folin Ciocalteu reagent. The elucidation was adjusted with distilled water to a final volume of 3ml and shaken thoroughly. The solution was incubated and kept in the dark placed and read at 760 nm was read against prepared blank. The total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight. The total sample was analysed in three replicates.

Estimation Total Flavonoid Content

Total Flavonoid content in *Moringa oleifera* (Lam.). whole plant extract was analysed by the aluminium chloride colorimetric system. 0.5ml of plant part extract of at different concentrations like 10µg, 20µg, 40 µg, 60 µg, 80 µg, and 100 µg were taken and the final volume was made up to 3ml with methanol. After that, 0.1ml AlCl3 (10%), 0.1ml of potassium acetate and 2.8ml of distilled water were added continuously and test solution was vigorously shaken. After 30 minutes for the incubation periods, absorbance was recorded at 415 nm. The concentration of flavonoids in test samples was calculated and expressed as the equivalent of quercetin (QE) / g of sample. The entire sample was analysed in three replicates.

RESULTS AND DISCUSSION

The qualitative phytochemical studies were carried out in the solvents viz. Methanol, Chloroform and n-Butanol. The Methanol, Chloroform and n-Butanol tuber and leaves extract of *Moringa oleifera* (Lam.). shows the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols.

Total content of Alkaloid, flavonoids and phenols in the Methanol, 1.926, 0.434 and 1.641 µg/ml. respectively, in the Chloroform 1.554, 0.391 and 0.856 µg/ml. and followed by in the n-Butanol leaves extract of *Moringa oleifera* (Lam.). that was 2.045, 0.423 and 0.426 µg/ml.

Table No. 1 Qualitative analysis of *Moringa oleifera* (Lam.) Leaves

Sr. No.	Phytochemical	Plant extract of Leaves		
		n-Butanol	Methanol	Chloroform
1	Alkaloids	++	++	+
2	Glycosides	++	+	+
3	Terpenoids	+	+	+
4	Steroids	++	++	+
5	Flavonoids	++	++	+
6	Saponins	++	+	++
7	Phenols	++	++	+
8	Tannins	++	-	+

Table. No. 2 Quantitative analysis of *Moringa oleifera* (Lam.) Leaves (µg/ml)

Sr.No.	Phytochemical	Plant extract of Leaves		
		n-Butanol	Methanol	Chloroform
1	Alkaloids	2.045	1.926	1.524
2	Flavonoids	0.423	0.434	0.391
3	Phenols	0.426	1.641	0.856

Results taken average of triplicates for different concentration of plant extract

Abideen *et al,* [1] studied on both aqueous and ethanolic extracts revealed the presence of the following phytochemical constituents saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids (aqueous extract) and tannins, saponins, flavonoid, steroids, cardiac glycosides, anthraquinones and alkaloids (ethanolic extract) in moringa leaves (Table 1). This is an indication that the Moringa leaves contained tannins, saponins, flavonoid, steroids, terpenoids, cardiac glycosides, anthraquinones and alkaloids as secondary metabolites. Several functions and roles are attributed to flavonoids in human and animals; this includes protection and fight against inflammatory disorders, allergies, diarrhea, microbes‟ invasion, platelet aggregation, ulcers, hepatotoxins, viruses, and tumours [11]. Flavonoids were able to achieved the aforementioned properties because of their antipyretic (fever-reducing), antioxidant, analgesic (pain-relieving), and spasmolytic (spasm-inhibiting) activities [10]. The presence of epicatechin, quercetin and luteolin in flavonoids plays pivotal roles in inhibition of fluids that is responsible for diarrhea [10]. The presence of flavonoids in Moringa leaves is responsible for it‟s used in acceleration of labour in south-western Nigeria during birth and this might be linked to high content of flavonoids and phenolic compounds [14]. Phenolic compounds are responsible for blockage of specific enzymes that causes inflammatory disorders. They also protect platelets from clumping through modification of the prostaglandin pathways [14]. As a result of the presence of phenolic compound in Moringa leaves, this is a signal that the Moringa leaves could act as antioxidants, anti-clothing agents, immune enhancers, antioxidants, and hormone modulators [15]. Moringa leaves can also be used as aphrodisiac because they are rich in phenolic compound which acts as stimulating agents [9].

CONCLUSION

Moringa oleifera (Lam.). is the rich source of phytochemicals, alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenol. Its extraction in n-butanol solvent shows highest intensity and content of phytochemicals followed by methanolic extract.

So, *Moringa oleifera* (Lam.). of plant presence different phytochemical compounds useful for Further studies of purification, identification and characterization of the active compounds of would be our priority in future studies.

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