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Research Article

**TLC, GC MS AND ANTIBACTERIAL STUDY OF METHANOL
EXTRACTS OF *TRIBULUS TERRESTRIS* THORNS AND
MORINAGA OLEIFERA FLOWERS**

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Running title: TLC, GC MS and antibacterial study of *Tribulus terrestris* and *Moringa oleifera*

Abstract:

The two medicinal plants namely, Moringa oleifera and Tribulus terrestris are well known for their medicinal roles ethnobotanically. The present work is to understand the presence of phytochemicals in each of them by TLC method and by GC MS analysis. The antibacterial study was also conducted for both the plants. The TLC profiles show the presence of terpenoids, steroids, flavonoids and alkaloids in both the plants. It was observed that antibacterial role of Tribulus terrestris was evident but Moringa flower extract did not show any antibacterial activity. The GC MS results show the presence of compounds such as Z-10-Tetradecen-1-ol acetate, Lyxitol, 1-O-hexyl-, 2-Butenoic acid, 2-methyl-, 2- (acetyloxy)- 1,1a,2,3,4,6,7,10,11,11adecahydro- 7,10-dihydroxy- 1,1,3,6,9-pentamethyl-4a,7aepoxy-5Hcyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR-[1aR,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*(E),11aS*]]- in Moringa flowers whereas molecules such as 2,2,4-Trimethyl-3-pentanol, n-Hexadecanoic acid and Z-1,6-Tridecadiene were present in Tribulus terrestris. Further work to understand the molecular mechanism of the compound found in the GC MS analysis is needed.*

Key words: *Moringa oleifera, Tribulus terrestris, GC MS, TLC, Antibacterial.*

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INTRODUCTION:

The knowledge of biomolecules present in medicinal plants is of great help in understanding the medicinal roles played by such plants. The claims made by ethnobotanical practitioner's world over can be authenticated by such knowledge since there is a dearth of information in this regard. Various methods are used to isolate, quantify and assess the phytochemicals present in plants. There are many reports on the phytochemical and GC MS analysis of plants and plant parts and their medicinal formulations [1-10]. The present study deals with the GC MS analysis, TLC study and antibacterial roles of two commonly used herbals plants, namely, *Moringa oleifera* flowers and *Tribulus terrestris* thorns.

Tribulus terrestris is an annual plant distributed in most parts of the world. It belongs to the family Zygophyllaceae. It has a variety of common names as goat's-head, caltrop, small caltrops, cat's-head, devil's eyelashes, devil's-thorn, devil's-weed, puncture vine, puncture vine, and tackweed. In Sanskrit it is known as "Gokshura" and in Tamil as "Nerinji". This plant is used in many traditional medications as in Ayurveda, Siddha Unani and Chinese. In all of the traditional medicines it is used as a diuretic and urinary tract infections. Fatima *et al* 2015 have elaborated in their review the various pharmacological activities of *Tribulus*. This plant has various medicinal applications such as diuretic (Jabbar *et al*, 2012), antitumor (Angelova *et al*, 2013), antibacterial and antifungal (Kianbakht and Jahaniani, 2003; Bayati and Al-molla, 2008), antioxidant (Dimitrova *et al*, 2012) and hypoglycemic (El-Tantawy and Hassanin, 2007) [11-17]. Various fractions of *Tribulus terrestris* is found to have aphrodisiac activity, Diuretic property, anti-urolithiatic, and anti-diabetic activity (Verma Priyanka *et al*, 2013). Fractions of *Tribulus terrestris* root and fruits is said to have anti-inflammatory property in albino rats (Ankeetha *et al*, 2017). Intake of *Tribulus terrestris* may help in diabetic nephropathy (Despande Vaishali Shailesh *et al*, 2017) [18-20].

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. The plant has common names like moringa, drumstick tree, horseradish tree, ben oil tree or benzoil tree. It is a fast-growing, drought-resistant tree, native to the southern foothills of the Himalayas in northwestern India and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. The bark, sap, roots,

leaves, seeds and flowers are used in traditional medicine. *Moringa* leaves have antioxidant, antistress and many other medicinal values (Naidu *et al*, 2012, Luqman and Kumar, 2011, Luqman *et al*, 2012, Anwar *et al*, 2007) [21-24]. *Moringa oleifera* is said to have anti-cancer and anti-diabetic properties (Gopalakrishna *et al*, 2016) [25]. 80% ethanolic extracts of *Moringa oleifera* flower inhibits the inflammation (Woan Sean Tan *et al*, 2015) [26]. The methanolic flower extract has a potential of natural preservative and a nutraceutical (Gull *et al*, 2016) [27]. Alcoholic and water extracts of *Moringa oleifera* seed, leaves and flower showed some antibacterial activity against some of the Human pathogens (Kafi and Elbir, 2014) [28].

The present deals with GC MS and TLC analysis of these *T. terrestris* thorns and *Moringa oleifera* flowers and also to find the antimicrobial roles of these two plant parts.

MATERIALS AND METHODS:**Preparation of Sample**

The plant parts, i.e. the thorns of *Tribulus terrestris* and flowers of *Moringa oleifera* were collected, dried and ground separately. The powers were soaked in methanol at 1:3 ration (W/v) for two days and the supernatants were collected after centrifugation. The supernatants were dried by evaporation and the resultant powders were kept in air tight condition till further process.

Thin Layer Chromatography of the samples

TLC method was used to know the presence of four important phytochemicals, namely, steroids, terpenoids, flavonoids and alkaloids in the samples.

Ten mg of each sample was taken and dissolved in the following mixtures to extract the respective phytochemical present therein as mentioned in the following list.

The solvent used for Steroids are Hexane & Ethyl Acetate in the ratio of 3:1. The solvent used for Terpenoids are Hexane & Acetic acid in the ratio of 9:1. The solvent used for Alkaloids are Toluene & Ethyl Acetate in the ratio of 4:1. The solvent used for Flavonoids are Toluene & Acetic acid in the ratio of 9:2.

A very small quantity of supernatant of each extract and also that of equal mixture of the two were charged on the TLC plate separately, with capillary tube and the sample was run for TLC in the chamber. The spots were visualized by keeping the TLC plates

in UV and in Iodine chambers. The plates were photographed and the Rf values were calculated. The standards were run parallel to find the variation in the sample.

Anti-Bacterial Test

50 mg of the powders both samples were taken and dissolved in 2 ml water separately. This was then serially diluted from the initial concentration to one-sixteenth of its original concentration. Muller-Hinton agar plate was prepared and *E. coli* was cultured using spread plate method. Wells were made in the

agar plates and samples were added to well at specific concentrations and incubated for 24 hrs. The plates were observed for microbial growth, photographed and the zone of clearance for each was measured.

RESULTS AND DISCUSSION:

Thin Layer Chromatography

The TLC profile of steroids, flavonoids, terpenoids and alkaloids are shown in Figure 1, 2, 3 and 4 and in the Rf values in Table 1 and 2.

The TLC profile for Steroid

STEROID was absent in the methanolic extract *Tribulus terrestris*, it was visualized in *Moringa oleifera* and in the mixed samples in UV chamber only with similar Rf value (Figure 1a and b)

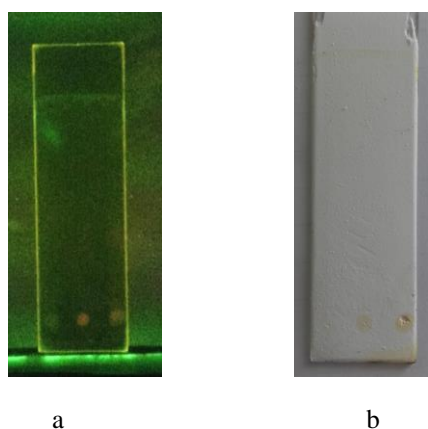


Fig. 1a and b: Indicating the presence of steroid In *T. terrestris* and *M. oleifera*

The TLC profile for FLAVONOIDS

The Rf values flavonoids of *Tribulus terrestris*, *Moringa oleifera* and the mixed samples were same as visualized in UV chamber whereas in the Iodine chamber the spots were visible only for *Tribulus terrestris* and mixed sample. Thus it seems that flavonoids present in *Moringa oleifera* could be different from that of the other two (Figure 2a and b).

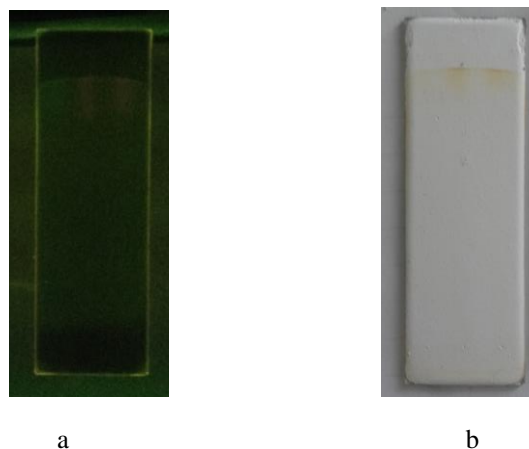


Figure 2 a and b: Indicating the presence of Flavonids In *T. terrestris* and *M. oleifera*

The TLC profile for TERPENOIDS

The terpenoid profile showed different spots for all the three, except for one spot which was present in both *Tribulus terrestris* and mixed sample. This may be due to the mixing of samples. (Figure 3a and b)

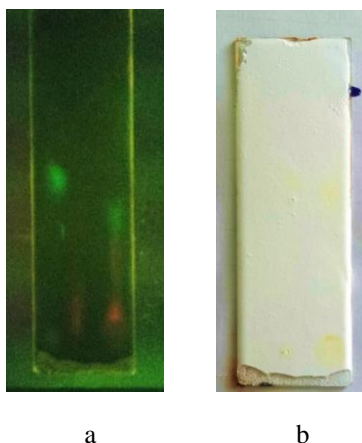


Figure 3 a and b: Indicating the presence of Terpenoids In *T. terrestris* and *M. oleifera*

The TLC profile for ALKALOIDS

The alkaloid TLC spots for *Tribulus terrestris* and mixed sample were the same whereas in *Moringa oleifera* there was only one spot corresponding to the other two (Figure 4 a and b)

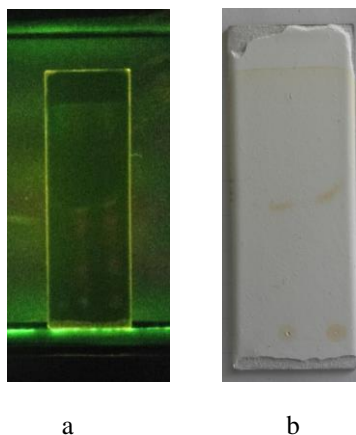


Figure 4a and b: Indicating the presence of steroid In *T. terrestris* and *M. oleifera*

In TLC the plates were viewed in UV and Iodine chamber. The iodine chamber did not show any spots for *Moringa oleifera* for any of the above parameters. The Rf values obtained for the samples are listed below

Table 1: Rf VALUES IN UV of different phytochemicals present in *T. terrestris*, *Moringa oleifera* and their mixture

TEST \ SAMPLE	STEROIDS	FLAVONOIDS	ALKALOIDS	TERPENOIDS
<i>Tribulus terrestris</i>	-	0.88	0.2, 0.45, 0.87	0.03, 0.2
<i>Moringa oleifera</i>	0.875	0.88	0.87	0.63
Mixed sample	0.875	0.88	0.2, 0.45, 0.87	0.03, 0.37

Table 2: Rf VALUES IN iodine chamber different phytochemicals present in *T. terrestris*, *Moringa oleifera* and there mixture

TEST	STEROIDS	FLAVONOIDS	ALKALOIDS	TERPENOIDS
<i>Tribulus terrestris</i>	-	0.93	0.45	0.57
<i>Moringa oleifera</i>	-	-	-	-
Mixed sample	-	0.93	0.53	0.53

GC MS Analysis Results

The GC MS analysis results are shown in Figure 5 a, b and c for *Moringa oleifera* and those of *Tibulus terrestris* in Figure 6 a, b and c. Table 3 and Table 4 indicate the GC MS details.

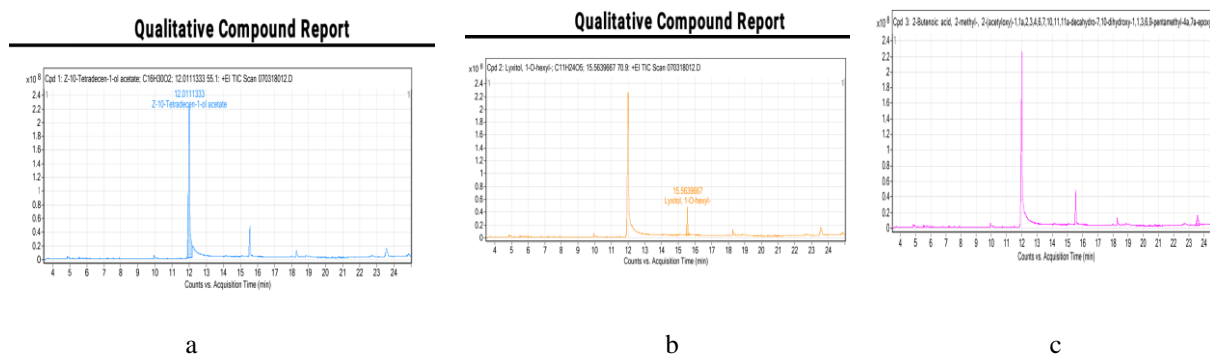


Figure 5 a, b and c: GC MS patterns of Methanolic extract of flowers of *Moringa oleifera* for each of the three molecules present.

Table 3: Indicates the GC MS profile of Moringa olifera flower with Retention time, possible type of compound, molecular structure, molecular weight, peak percentage and reported medicinal values each compound.

Sl. No	Retention Time	Compound Name	Mol structure	Mol Weight	Peak Percentage	Medicinal Role
1.	12.01	Z-10-Tetradecen-1-ol acetate	C16H30O2	254	82.96	Increase Zinc bioavailability, provides Zinc, Ologosaccharide provider
2.	15.56	Lyxitol, 1-O-hexyl-	C11H24O5	236	10.65	Aldehyde oxidatse inhibitor, Anticancer, antidote, antitumor, catechol O methyl transferase inhibitor, Decrease Glutamate Oxaloacetate Transaminase, Decrease oxalate excretion, , Decrease Oxalate, Down regulation of nuclear and cytosol androgen, Increase Osteocalcin, Inhibit Destruction of Glycosaminoglycans, Inhibit Production of Tumor Necrosis Factor, Inhibit Production of Uric Acid, NADH-Oxidase-Inhibitor, NADH-Oxidase-Inhibitor.
3.	23.56	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-, 1,1a,2,3,4,6,7,10,11,11a-decahydr o-7,10-dihydroxy-1,1,3,6,9-pentamethyl-4a,7aepoxy-5Hcyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR][1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*(E),11aS*]]-	C27H38O8	440	6.40	Not Known

It is observed from the GC MS analysis of *Moringa oleifera* methanolic extract that three biomolecules were found to be present viz. Z-10-Tetradecen-1-ol acetate (82.96%), Lyxitol, 1-O-hexyl- (10.65%) and 2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydroxy-1,1,3,6,9-pentamethyl-4a,7a-epoxy-5Hcyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR-[1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*(E),11aS.

Z-10-Tetradecen-1-ol acetate (82.96%) which is present in large quantity increase Zinc bioavailability, provides Zinc and is an oligosaccharide provider. The activities of Lyxitol, 1-O-hexyl- (10.65%) is mentioned in table which indicates its importance as a medicine. The medicinal properties of *Moringa* as mentioned earlier match well with the activities of the compounds as presented in the GC MS analysis results.

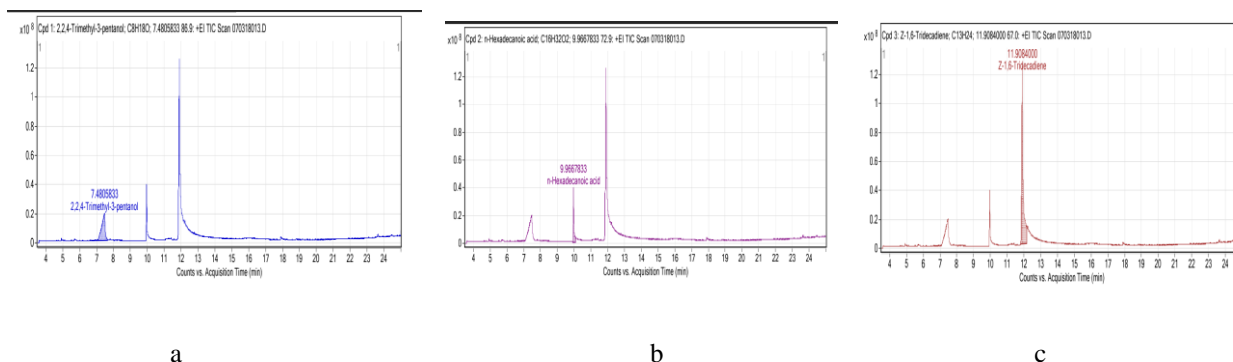


Fig. 6: a, b and c. GC MS patterns of Methanolic extract of thorns of *Tribulus terrestris* for each of the three molecules present.

Table 4: Indicates the GC MS profile of *Tribulus terrestris* thorns with Retention time, possible type of compound, molecular structure, molecular weight, peak percentage and reported medicinal values each compound.

Sl. No	Retention Time	Compound Name	Mol structure	Mol Weight	Peak Percentage	Medicinal Role
1.	7.48	2,2,4-Trimethyl-3-pentanol	C ₈ H ₁₈ O	130	23.58	Not known
2.	9.97	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	9.77	Acidifier, Arachidonic acid inhibitor, inhibit production of uric acid, increase aromatic amino acid decarboxylase activity, anaphylactic, antitumor, Aryl amine-N-Acetyl transferase inhibitor, decrease nor- epinephrine production, down regulate nuclear and cytosolic androgens, GABA-nergic, Increase natural killer cell activity, inhibit production of tumor necrosis factor, myoneural stimulant. antioxidant, hypocholesterolemic, nematicide, antiandrogenic, flavoring agents, hemolytic, antibacterial and cytotoxic, 5-alpha reductase inhibitor.
3.	23.56	Z-1,6-Tridecadiene	C ₁₃ H ₂₄	180	66.66	Provides Zinc, increases zinc bioavailability

The GC MS results of *Tribulus terrestris* show three components viz. 2,2,4-Trimethyl-3-pentanol (23.58%), n-Hexadecanoic acid (9.77%) and Z-1,6-Tridecadiene (66.66%). 2,2,4-Trimethyl-3-pentanol although present in high quantity there are no known reports of medicinal role. n-Hexadecanoic acid is a known medicinal molecule with various activities Rajeswari *et al*, 2013; Sudarshan *et al*, 2010; Sharma *et al*, 2011; Gazali *et al*, 2014. Z-1, 6-Tridecadiene is also a molecule that increases the bioavailability of Zinc, a Zinc provider and also an oligoachharide provider [29-32].

It is interesting to find the major molecules present in both *Moringa* and *Tribulus* are common in providing

Zinc and increasing the bioavailability of Zinc. These two plant and plant parts are used separately and also in combination to treat prostate enlargement and prostate cancer. It could be an interesting work to understand the mechanism of action of these two plants at phytochemical and molecular level to prove their efficacy in the cure of many diseases as claimed by traditional medicinal practice.

Anti-Bacterial Activity

The antibacterial study of *Tribulus terrestris* and *Moringa oleifera* was studied on *E. coli* and the results are indicated in Figure 7, 8 and 9.



Fig.7: Anti-bacterial activity of *Moringa oleifera* on *E. coli*



Fig.8: Anti-bacterial activity of *Tribulus terrestris* on *E. coli*



Fig.9: Anti-bacterial activity of mixed sample on *E. coli*

The anti-bacterial activity of *Tribulus terrestris* on *E. coli* was found in the initial and the one-fourth concentrations. The mixed sample showed anti-bacterial activity in the initial, half and one-fourth concentrations. But highest activity was observed in one-fourth concentration of the mixed sample. No anti-bacterial activity was observed in *Moringa oleifera* sample. Thus in the present study it was observed that *Tribulus* did show some antibacterial activity but *Moringa* flowers did not show any antimicrobial activity.

CONCLUSIONS:

From the above study it was observed that the four important secondary metabolites are present in both these plant parts indicating their medicinal role. The presence of some important compounds as seen in the GC MS study indicate that further study is warranted to prove the medicinal roles of these plants in light of the knowledge already gathered. The antibiotic role of *Tribulus* is clearly shown whereas the flowers of *Moringa* did not show any antibiotic role.

COMPETING INTERESTS

This is to inform that no conflict of interest exist among the author.

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