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Phytochemical Investigations and Biological Potential of *Moringa oleifera* Pods

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Fresh pods of *Moringa oleifera* were collected, shadow-dried and chopped into small pieces. These were then extracted using hot methanol by refluxing method. The obtained methanolic extract was divided into two parts. One part was mixed with silica gel (60-120 mesh size) and subjected to column chromatography which on elution with solvents of increasing polarity and their mixtures afforded five compounds. These isolated compounds were characterized as 22-tritetracontanone, 1-octacosanol, methyl tetratricontanoate, hexadecane and β-sitosterol with the help of spectroscopic and analytical techniques. Other part of the methanolic extract was further fractionated with different solvents *viz*., hexane, benzene, chloroform, ethyl acetate, acetone and water. These fractions and methanolic extract were screened for antifungal activity by poisoned food technique at 250, 500, 1000 and 2000 µg/mL concentrations against two phytopathogenic fungi. All the extract/fractions were more toxic against *Rhizoctonia solani* than *Fusarium oxysporum*.

Keywords: *Moringa oleifera*, Antifungal activity, Isolation, Phytochemicals, *Rhizoctonia solani*, *Fusarium oxysporum*.

INTRODUCTION

Agriculture is the largest sector which plays a significant role in the socio-economic development of India. But the yield of crops is declining because of different pests like weeds, fungi and insects. So, in order to control these pests, synthetic pesticides are widely used [1] which lead to a number of ecological problems such as environmental pollution, left over residues, toxicity to humans and other warm blooded animals [2], *etc*. Botanical pesticides can be a great alternative to these synthetic pesticides and can help us to overcome on these pesticides. *Moringa oleifera* is a shrub and small deciduous tree native to western and Himalayan regions, India, Pakistan, Africa and Arabia [3]. It is referred by a number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree and Mother's best friend. It is known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil [4]. All parts of *Moringa oleifera* are edible and possess various medicinal properties like anti-inflammatory, antiulcerative [5], antihypertensive [6], antioxidant [7], antibacterial and antifungal activity [7]. The pods of *Moringa oleifera* are fibrous (46.78 %) and are valuable to treat digestive problems. These can be eaten raw or pickled or can be cooked from the time they first appear up to they grow about 30 cm long and become brown in colour. Immature pods are good source of palmitic acid, linoleic acid, linolenic acid and oleic acids. So they can be used in the diet of an obese person [8]. The seeds help to cure eye diseases, head complaints, hyperthyroidism, Chrohn's disease and sexually transmitted diseases. They can also be used as a relaxant for epilepsy. As the seeds possess antibiotic and anti-inflammatory properties, they can be used to treat arthritis, rheumatism and gout cramps [9]. *Moringa* seeds contain 19-47 % oil which is highly valuable and is used in cosmetics, soaps and perfumes because of its absorbing powers and retaining odor properties. Seeds also act as a natural coagulant having a cationic protein which helps in removing turbidity of water and reducing bacterial contamination from water [10]. Shelled *Moringa* seeds possess a great potential to eliminate toxic metals like cadmium from water resource by forming an amino acid-Cd interaction [11]. In view of various activities and medicinal properties shown by pods of this plant and our search for natural agrochemicals of plant origin, the present study involves the isolation and characterization of compounds from pods of *Moringa oleifera* and evaluation of its various extracts/fractions for antifungal activity.

EXPERIMENTAL

All the solvents used were of analytical grade. Melting points were determined with a Ganson electrical melting point

apparatus. IR spectra were recorded with a Perkin Elmer Spectrum RX-I FTIR. It has a resolution of 1cm^{-1} and scan range of 4000 to 250 cm^{-1}. ^{1}H NMR spectra of the isolated compounds were recorded on sophisticated multinuclear FT NMR Spectrophotometer model Avance-II (Bruker 400 MHz). $CDCl_3$ and DMSO were used as solvents. Chemical shifts were recorded in δ (ppm) using tetramethyl silane (TMS) as an internal standard. LC-MS were recorded with a Waters Micromass Q-ToF micro mass spectrometer. It is equipped with electron spray ionization (ESI) and atmospheric pressure chemical ionization (APcI) source having mass range of 4000 amu in quadruple and 20000 amu in ToF.

Pods of *Moringa oleifera* were collected from the campus of Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. These were washed thoroughly with water to remove dust, shadow dried and chopped into small pieces. These were then kept in air tight containers for further use.

Extraction and fractionation: The shadow dried chopped pieces of pods of drumstick tree were extracted with hot methanol by refluxing method for 8 h. The process was repeated thrice and the respective extractives were pooled together. The obtained extractives were evaporated on a rotary evaporator to give a crude extract. This extract was further divided into two parts. One major part was mixed with silica gel (60-120 mesh size) and used to fill the column. The remaining part was further fractionated with different polarity solvents *viz*. hexane, benzene, chloroform, ethyl acetate, acetone and water. Each fraction was evaporated to give a crude mass and stored in a refrigerator till use. These fractions and methanolic extract were used for determination of antifungal activity (Fig. 1).

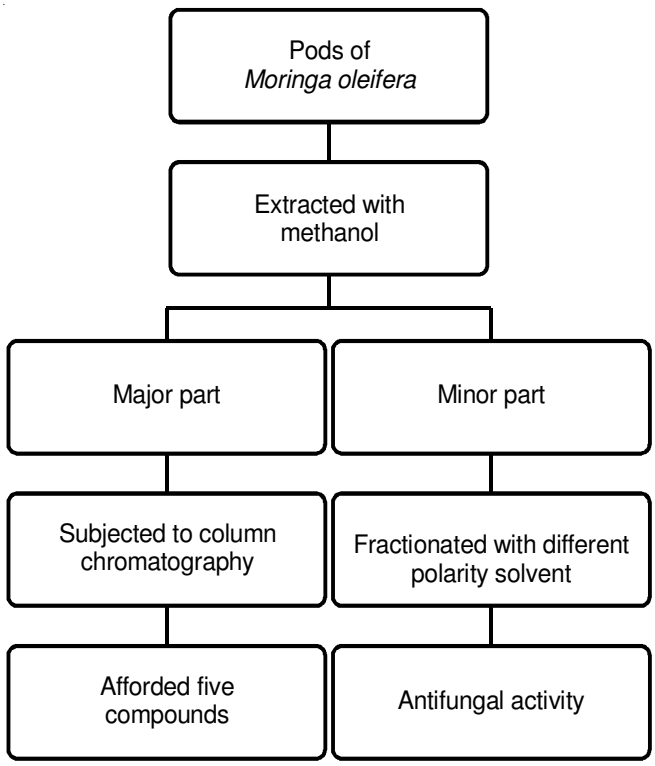

Fig. 1. Extraction, fractionation and isolation scheme of pods of *Moringa oleifera*

Preliminary phytochemical screening: The freshly prepared methanolic extract of pods of *Moringa oleifera* was subjected to qualitative chemical tests using standard methods. This helps in the identification of various classes of bioactive chemical constituents.

Column chromatography: The methanolic extract of pods of *Moringa oleifera* was mixed with silica gel 60-120 mesh size subjected to column chromatography. A glass column of 1000 × 40 mm size was packed with slurry of silica gel (60-120 mesh size) in hexane. A portion of the methanolic extract of pods was introduced onto the column and eluted with solvents of increasing polarity. The elutropic series of solvents used was hexane, benzene, ethyl acetate, methanol and their mixtures. Each eluate obtained was monitored by using thin layer chromatography plates. The column chromatography afforded five compounds labeled as 1 to 5.

Compound **1** was isolated on elution with benzene:hexane (1:19) and crystallized out from benzene to get white solid, 30 mg, m.p.: 94-96 °C. Its R_f value was found to be 0.36 in benzene:hexane (3:7). The molecular formula $C_{43}H_{86}O$ was deduced from 620 $(M+2)^+$ peak by its LC-MS. ^{1}H NMR (δ, $CDCl_3$) 0.89 (m, *J* = 4.0 Hz, 6H, 2×-C**H**$_3$), 1.25 (br, 72H, 36×-C**H**$_2$), 1.61 (m, *J* = 8.0 Hz, 4H, 2×-C**H**$_2$CH$_2$CO), 2.35 (m, *J* = 4.0 Hz, 2×-C**H**$_2$CO), IR (KBr, ν_{max}, cm^{-1}): 2917, 2849, 1706, 1463, 1300, 930, 719, LC-MS (*m/z*, % intensity) 620 (3.04), 595 (14.26), 551 (13.95), 507 (10.92), 468 (21.84), 409 (100), 387 (42.69), 316 (25.57), 301 (30.13), 202 (58.71).

Compound **2** was a white crystalline (30 mg) with m.p.: 81-84 °C. It was obtained on elution with benzene:hexane (1:14) and recyrstallized from benzene:hexane (1:1). Its R_f value was found to be 0.21 in benzene:hexane (1:1). The molecular formula $C_{27}H_{58}O$ was deduced from *m/z* 410 M^+ peak by its LC-MS. It gave positive test to James reagent to prove the presence of primary alcohol. ^{1}H NMR (δ, $CDCl_3$) 0.86 (t, *J* = 8.0 Hz, 3H, 1× -C**H**$_3$), 1.25-1.31 (m, *J* = Hz, 52H, 26× -C**H**$_2$), 2.40 (s, *J* = Hz, 1H, 1× -CH$_2$-O**H**), 3.62 (t, *J* = 8.0 Hz, 2H, 1× -C**H**$_2$-OH), IR (KBr, ν_{max}, cm^{-1}): 3323, 2955, 2849, 2917, 1473, 1463, 1062, 730, LC-MS (*m/z*, % intensity) M^+ 410 (2.8), 387 (20.66), 316 (5.83), 302 (7.96), 261 (5.83), 240 (13.99), 238 (100), 202 (28.08), 162 (3.45), 104 (5.10), 83 (11.95).

Compound **3** was obtained as a pale yellow solid (31 mg) on elution with benzene:hexane (1:9). Its R_f value was found to be 0.17 in benzene:hexane (1:1). The hydroxamic acid test for this compound confirmed the presence of ester. Its molecular formula $C_{35}H_{70}O_2$ was deduced from *m/z* 523 $(M+1)^+$ by its LC-MS. ^{1}H NMR (δ, DMSO) 0.89 (t, *J* = 8.0 Hz, 3H, 1× -C**H**$_3$), 1.25 (br, 32H, 16× -C**H**$_2$), 1.49 (t, *J* = 8.0 Hz, 2H, 1× -C**H**$_2$-CO), 3.49 (s, *J* = 4.0 Hz, 3H, 1× -OC**H**$_3$), IR (KBr, ν_{max}, cm^{-1}): 2955, 2917, 2849, 1743, 1472, 1462, 1072, 729, 719, LC-MS (*m/z*, % intensity) M^+ 523 (3.00), 507 (7.39), 468 (92.68), 440 (39.75), 409 (15.66), 387 (4.24), 365 (6.95), 316 (26.79), 301 (100), 239 (14.35), 224 (4.75), 202 (82.58), 168 (3.29), 148 (5.05), 123 (6.73), 106 (90.77), 101 (9.81).

Compound **4** was obtained as viscous oil (20 mg) on elution with benzene:hexane (1:1). Its R_f value was found to be 0.24 in ethyl acetate: benzene (1:14). The molecular formula $C_{16}H_{34}$ was deduced from its LC-MS. ^{1}H NMR (δ, $CDCl_3$) 0.87 (m, *J* = 4.0 Hz, 6H, 2× -C**H**$_3$), 1.25-1.29 (br, *J* = 16.0 Hz, 28H, 14×

-C**H**$_2$), IR (cm^{-1}); 2917, 2849, 1463, 1261, 1094, LC-MS (*m/z*, % intensity) 316 (23.78), 301 (100), 279 (14.06), 243 (10.30), 202 (29.80), 163 (3.03), 149 (3.96).

Compound **5** was obtained on elution with ethyl acetate: benzene (1:1) and crystallized out from ethyl acetate, 35 mg, m.p. 135-137 °C. It gave green colour with Liebermann Burchard reaction indicating the presence of steroid. LC-MS and elemental analysis suggests the molecular formula and mass to be $C_{29}H_{45}O$ and 412. ^{1}H NMR (δ, DMSO) 0.67 (s, *J* = 8.0 Hz, 3H, 1×C_{18}-C**H**$_3$), 0.79 (d, *J* = 4.0 Hz, 6H, 1×C_{26}-C**H**$_3$ and 1×C_{27}-C**H**$_3$), 0.83 (t, *J* = 8.0 Hz, 3H, 1×C_{29}-C**H**$_3$), 0.90 (d, *J* = 4.0 Hz, 3H, 1×C_{21}-C**H**$_3$), 1.24 (s, 3H, 1×C_{19}-C**H**$_3$), 1.40-3.18 (m, 29H, 11× -C**H**$_2$ and 7× -C**H**), 5.12 (m, 1H, -O**H**), 5.32 (br, *J* = 4.0 Hz, 1H, C_6-C**H**), IR (cm^{-1}); 3400, 2934, 2869, 1463, 1367, 1074, 925, LC-MS (*m/z*, % intensity) 409 (10.56), 375 (3.78), 301 (20.83), 243 (16.05), 154 (7.35), 123 (23.46), 106 (100).

Bioevaluation

Test organism: The antifungal activity of different extracts/fractions of pods of *Moringa oleifera* was investigated using two phytopathogenic fungi *i.e. Rhizoctonia solani* and *Fusarium oxysporum* which were obtained from the Department of Plant Pathology, College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. The fungal isolates were allowed to grow on potato dextrose agar (PDA) [12] at 25 ± 2 °C until they sporulated. A 4-6 day old culture of each fungus was used for testing antifungal activity.

Bioassay: Poisoned food technique [13] was used for determination of antifungal activity of different solvent fractions and extract of *Moringa oleifera* pods. Two sets were maintained-one for the treatment and another for control. The treatment set at different concentrations *viz*. 250, 500, 1000 and 2000 µg/mL was prepared by mixing the required quantity (25, 50, 100 and 200 mg respectively) in 1 mL of DMSO and then added pre-sterilized PDA. In control set, 1 mL DMSO was mixed with PDA. These treatments and control were then poured in pre-sterilized petri plates and allowed to solidify at room temperature. After solidification, mycelia disc of 5 mm diameter cut out from 4-6 day old culture of test fungi were aseptically placed in petri plates of different treatment and control sets. The petri plates were then wrapped with para film along the rim to prevent contamination. The inoculated plates were then inverted and incubated at 25 ± 2 °C and the observations were recorded when the control plate got completely filled with test fungus. Colony diameter was determined by measuring the average radial growth of each plate. The data recorded in each case was mean of three replicates. The fungal growth inhibition (%) was calculated by using the following formula:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where C = mycelia growth in control plate, T = mycelia growth in treated plate.

The concentration of plant extract/fractions producing 50 % growth inhibition (EC_{50}) was calculated using SPSS statistics 19 software.

Data analysis: All the experimental measurements were carried out in triplicate and results were presented as mean ± standard deviation. One way and two way analysis of variance (ANOVA) was carried out to assess any significant differences between the means ($p < 0.05$) in Online Statistical Analysis (OPSTAT), CCS HAU, Hisar. EC_{50} values of antifungal activity were calculated using SPSS statistics 19 software. All other measurements and calculations were carried out in Microsoft Excel 2007.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: The data presented in Table-1 revealed that saponins, carbohydrates, anthraquinone glycosides, alkaloids, flavonoids, terpenoids, phytosterols, proteins and amino acids were present in the methanolic extract of pods of *Moringa oleifera* while tannins and cardiac glycosides were absent.

TABLE-1
PRELIMINARY PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF PODS OF *Moringa oleifera*

Phytochemicals tested	Name of the test	Result
Saponins	Frothing test	+
Tannins	Ferric chloride test	–
Carbohydrates	Fehling's test, Tollen's reagent test	+
Cardiac glycosides	Keller-Killiani test	–
Anthraquinone glycosides	Hydroxyanthraquinine test	+
Alkaloids	Hager's test	+
Flavonoids	Alkaline reagent test	+
Terpenoids	Salkowski test	+
Phytosterols	Liebermann-Burchard's test	+
Protein	Biuret test	+
Amino acids	Millon's test	+

+ shows the presence while; – shows the absence

Isolation and characterization of isolated compounds:

Compound 1 (22-tritetracontanone): Methanolic extract of pods of *Moringa oleifera* on silica gel (60-120 mesh size) column chromatography gave compound **1** in benzene:hexane (1:19) solvent system. It was recrystallized in benzene (30 mg), m.p.: 94-96 °C (literature m.p. 96.1-96.3 °C) [14]. Its R_f value was found to be 0.36 in benzene:hexane (3:7). Its molecular formula $C_{43}H_{86}O$ was deduced from LC-MS, 620 $(M+2)^+$ peak by its LC-MS. Absorptions at 1706 cm^{-1} confirmed the presence of >C=O group in this compound. Other absorptions were at 2917, 2849, 1463, 1300, 930 and 719 cm^{-1}.

The ^{1}H NMR spectra of this compound in $CDCl_3$ exhibited a multiplet at 0.89 δ integrating for two terminal methyl groups. A broad signal at 1.25 δ integrating for seventy-two protons indicates the presence of thirty six methylene groups. Another multiplet appeared at 1.61 δ with *J* = 8.0 Hz indicated the presence of two methylene groups at position beta to ketonic groups. Two protons of methylene group attached to >C=O group appeared at 2.35 δ with *J* = 4.0 Hz. The spectral data analysis and elemental analysis is in perfect assignment with the literature data [14] of 22-tritetracontanone (Fig. 2). From the literature survey, it seems that this is the first time report of isolation and characterization of 22-tritetracontanone from pods of *M. oleifera*.

Compound 2 (1-octacosanol): Compound 1-octacosanol (Fig. 2) was obtained on elution with benzene:hexane (1:14)

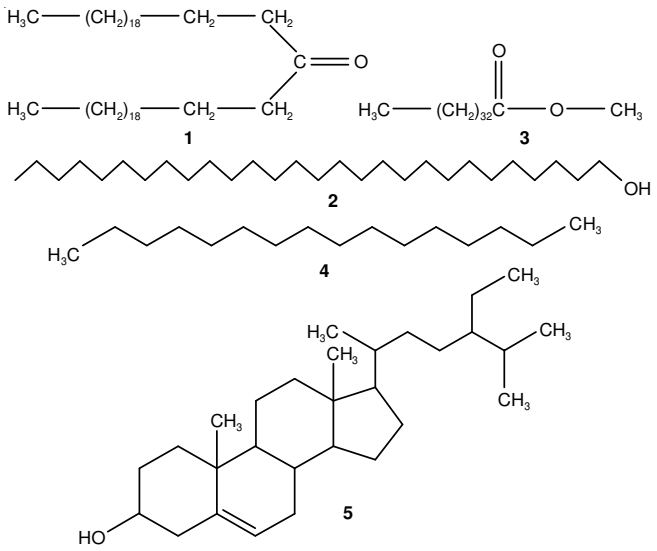

Fig. 2. Chemical structures of compounds isolated from pods of *Moringa oleifera*

and crystallized from benzene:hexane (1:1) having melting point 81-84 °C. Its R_f value was found to be 0.21 in benzene: hexane (1:1). The IR absorption at 3323, 2955, 2917, 2849, 1473, 1463, 1062 and 730 cm^{-1} indicated the presence of –OH and $–CH_2$ groups. M^+ peak of LC-MS provides information regarding molecular mass of compound **2** to be 410 and molecular formula to be $C_{28}H_{58}O$.

The ^1H NMR spectra of this compound in $CDCl_3$ displayed a triplet for terminal methyl group at 0.86 δ. Other methylene groups appeared as multiplet in the range of 1.25-1.31 δ. Methylene groups attached to hydroxyl group appeared at 3.62 δ. ^1H NMR spectra displayed a singlet at 2.40 δ integrating for one proton confirmed the presence of –OH group. This confirmed the possibility of 1-octacosanol.

Compound 3 (methyl tetratriacontanoate): It was obtained on elution with benzene:hexane (1:9) as a solid (31 mg). It was crystallized from benzene:hexane (1:1). The presence of ester was determined by hydroxamic test. The melting point of the compound was found to be 74-76 °C (literature m.p. 83.2-83.4 °C) [15]. Its purity was checked by TLC which gave a single deep yellow spot (R_f = 0.17) on development with iodine. The IR spectra of this compound gives absorption bands at 2955, 2917, 2849, 1743, 1472, 1462, 1072, 719 cm^{-1} indicating the presence of >C=O and $–CH_2$ group. LC-MS analysis suggested that the molecular mass and molecular formula of this compound to be 522 and $C_{35}H_{70}O_2$.

The ^1H NMR spectrum of compound **3** in DMSO displayed a triplet for terminal methyl group at 0.89 δ with *J* value 8.0 Hz. There was a broad signal for thirty two protons at 1.25 δ indicating the presence of sixteen methylene groups. Protons of methylene group attached to >C=O group appeared as triplet at 1.49 δ integrating for two protons. A singlet centered at 3.49 δ for three protons terminal to >C=O functionality. Thus, compound 3 could be characterized as methyl tetratricontanoate (Fig. 2). This is the first time report of isolation and characterization of methyl tetratricontanoate from *Moringa oleifera* pods.

Compound 4 (hexadecane): Fractions 1-30 were repeatedly separated by silica gel column chromatography on elution with benzene:hexane (1:1). The purity of the compound was confirmed on the basis of its behaviour on TLC plate. It was obtained as a deep yellow spot on TLC plate with iodine as developing phase. Its R_f value was found to be 0.24 in ethyl acetate: benzene (1:14). The physical state of the compound was found to be oily viscous. LC-MS data showed that the molecular formula of the compound 4 is $C_{16}H_{34}$ with molecular mass 226.

The IR spectra of this compound gave no signal for carbonyl and hydroxyl group indicating the compound to be aliphatic in nature. Absorption peaks at 2917, 2849, 1463, 1261 and 1094 cm^{-1} are characteristic peaks of methylene groups. The ^1H NMR spectra of compound **4** in $CDCl_3$ exhibited a multiplet at 0.87 δ integrating for six protons of two terminal methyl groups with coupling constant *J* = 4.0 Hz. All the fourteen methylene groups resonating at 1.25-1.29 δ appeared as a broad signal indicates the presence of twenty eight protons. A complete agreement of the data of the compound **4** with the literature data of hexadecane established the identity of the compound **4** to be hexadecane (Fig. 2).

Compound 5 (β-sitosterol): The compound was obtained on elution with ethyl acetate: benzene (1:1) and crystallized out from ethyl acetate, 35 mg, melting point 135-137 °C (literature m.p. 136-137 °C) [16]. It responded to Liebermann-Burchard reaction and gave green colour indicating the presence of steroid. The IR spectra of this compound showed a peak at 3400 cm^{-1} indicating the presence of –OH group in the compound. The LC-MS and elemental analysis suggests the molecular formula and mass to be $C_{29}H_{45}O$ and 412.

The ^1H NMR spectra of this compound in DMSO exhibited a singlet at 0.67 δ for three protons which was assignable to methyl group present at C_{18} position. A doublet centered at 0.79 δ with *J* = 4.0 Hz integrating for six protons indicated two methyl groups positioned at C_{26} and C_{27}. A doublet centered at 0.90 δ with *J* = 4.0 Hz integrating three protons suggested the presence of a methyl group at C_{21} and a triplet at 0.83 δ (*J* = 8.0 Hz) representing three protons was assignable to methyl group at C_{29} position. A singlet at 1.24 δ representing three protons could be due to methyl group at C_{19} position. Appearance of a multiplet in the range of 1.40-3.18 δ representing twenty-nine protons hinted the presence of seven methines and eleven methylenes. Another multiplet centered at 5.12 δ integrating for one proton could be of a proton positioned at alpha to hydroxyl group. A broad signal at 5.32 δ for one proton was assigned to an olefinic proton. A complete agreement of the data of compound 5 with the literature data of β-sitosterol established the identity of the compound 5 to be β-sitosterol (Fig. 2). This is an already reported compound from pods of *Moringa oleifera*.

Bioevaluation: Antifungal activity of different extract fractions of pods of *Moringa oleifera* was determined by using poisoned food technique. The data given in Table-2 has shown that the acetone fraction of pods of *M. oleifera* possessed maximum activity with 67.65 ± 1.18 % inhibition against *Rhizoctonia solani* fungus at 2000 μg/mL concentration. It was found to be the most active with 1448.94 μg/mL EC_{50} value. Methanol extract also exhibited good activity having 60.20 ± 0.34 % mycelia growth inhibition at 2000 μg/mL concentration followed by ethyl acetate fraction with 54.12 ± 0.59 % inhi-

bition. Moderate activity was shown by chloroform and benzene fractions with 46.47 ± 0.59 % and 44.71 ± 1.18 % antifungal activity at the highest tested concentration (2000 µg/mL) respectively. Minimum activity was found to be shown by water fraction having 40.20 ± 0.90 % growth inhibition at 2000 µg/mL concentration against *R. solani*. A study of the data shown in Table-3 revealed that maximum growth inhibition (32.55 ± 0.34 %) against *Fusarium oxysporum* was exhibited by benzene fraction of pods of *M. oleifera* at 2000 µg/mL concentration. Comparatively, moderate activity was shown by chloroform and ethyl acetate fractions with 22.94 ± 0.59 % and 21.37 ± 0.34 % inhibition at 2000 µg/mL respectively against the test organism. Water fraction and methanol extract possessed low activity having 16.47 ± 1.18 % and 16.86 ± 0.34 % mycelia growth inhibition at 2000 µg/mL concentration. Hexane fraction exhibited 14.51 ± 0.34 % inhibition and acetone fraction exhibited lowest inhibition *i.e.* 13.14 ± 0.34 % at 2000 µg/mL concentration against *F. oxysporum* fungus.

A perusal of activity data (Figs. 3 and 4) revealed that 2000 µg/mL concentration was found to be most toxic while 250 µg/mL concentration was found to be least toxic. Irrespective of concentrations, all the extract/fractions were found to be more toxic against *Rhizoctonia solani* than *Fusarium oxysporum*. However, all the concentrations were significantly different from one another. Critical difference values for antifungal activity of various extract/fractions of pods of *Moringa oleifera* were calculated. Interaction of compounds and concentrations was statistically significant with a value 1.042 against *R. solani* and 0.753 against *F. oxysporum* for concentration × compound.

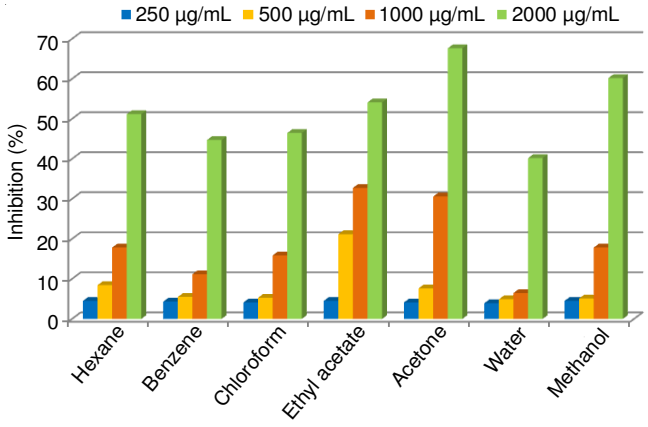

Fig. 3. Comparative analysis of antifungal activity of different extract/fractions of pods of *Moringa oleifera* against *Rhizoctonia solani*

TABLE-2
ANTIFUNGAL ACTIVITY (%) AND EC_{50} VALUES (µg/mL) OF VARIOUS EXTRACT/FRACTIONS OF PODS OF *Moringa oleifera* AGAINST *Rhizoctonia solani*

Extract/Fractions	Growth inhibition (%)				EC_{50} (µg/mL)
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL	
Hexane	4.51 ± 0.34	8.43 ± 0.68	17.84 ± 0.68	51.18 ± 0.59	2208.31
Benzene	4.31 ± 0.34	5.49 ± 0.34	11.18 ± 0.59	44.71 ± 1.18	2788.40
Chloroform	4.12 ± 0.59	5.29 ± 0.59	15.88 ± 1.18	46.47 ± 0.59	2484.97
Ethyl acetate	4.51 ± 0.34	21.18 ± 0.59	32.75 ± 0.34	54.12 ± 0.59	1704.63
Acetone	4.12 ± 0.59	7.65 ± 0.59	30.59 ± 0.59	67.65 ± 1.18	1448.94
Water	3.92 ± 0.34	4.90 ± 0.34	6.47 ± 0.59	40.20 ± 0.90	3347.42
Methanol	4.51 ± 0.34	5.10 ± 0.68	17.84 ± 0.34	60.20 ± 0.34	1833.91
Factors	SE (d)		CD at 5 %		
Concentration	0.196		0.394		
Compound	0.259		0.521		
Conc. × Compound	0.519		1.042		

All the values are mean ± S.D.; Mean of three replicates was taken (n = 3); EC_{50} means inhibition concentration at which 50 % of the growth is inhibited.

TABLE-3
ANTIFUNGAL ACTIVITY (%) AND EC_{50} VALUES (µg/mL) OF VARIOUS EXTRACT/FRACTIONS OF PODS OF *Moringa oleifera* AGAINST *Fusarium oxysporum*

Extract/Fractions	Growth inhibition (%)				EC_{50} (µg/mL)
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL	
Hexane	3.73 ± 0.34	10.00 ± 0.59	11.96 ± 0.34	14.51 ± 0.34	6574.73
Benzene	6.27 ± 0.68	9.61 ± 0.34	30.78 ± 0.34	32.55 ± 0.34	3638.26
Chloroform	9.61 ± 0.34	17.45 ± 0.34	19.61 ± 0.34	22.94 ± 0.59	7348.36
Ethyl acetate	4.51 ± 0.34	6.08 ± 0.34	15.49 ± 0.90	21.37 ± 0.34	7871.26
Acetone	3.73 ± 0.34	8.63 ± 0.34	11.96 ± 0.34	13.14 ± 0.34	12627.35
Water	3.73 ± 0.34	9.61 ± 0.34	13.73 ± 0.34	16.47 ± 1.18	9165.64
Methanol	4.51 ± 0.34	6.47 ± 0.00	11.37 ± 0.34	16.86 ± 0.34	9624.01
Factors	SE (d)		CD at 5 %		
Concentration	0.142		0.284		
Compound	0.187		0.376		
Conc. × Compound	0.375		0.753		

All the values are mean ± S.D.; Mean of three replicates was taken (n = 3); EC_{50} means inhibition concentration at which 50 % of the growth is inhibited.

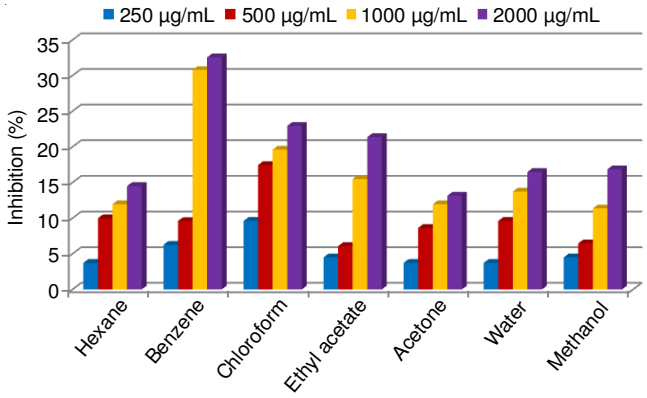

Fig. 4. Comparative analysis of antifungal activity of different extract/fractions of pods of *Moringa oleifera* against *Fusarium oxysporum*

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