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Assessment of Phytochemicals and Antioxidant activity of *Moringa Oleifera* leaves and Stem Bark

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Abstract: *Moringa oleifera* Lam., also known as the 'drumstick tree,' is recognized as a vibrant and affordable source of phytochemicals, having potential applications in medicines, functional food preparations, water purification, and biodiesel production. The multiple biological activities including antiproliferation, hepatoprotective, anti-inflammatory, antinociceptive, antiatherosclerotic, oxidative DNA damage protective, antiperoxidative, cardioprotective, as well as folk medicinal uses of *M. oleifera* (MO) are attributed to the presence of functional bioactive compounds, such as phenolic acids, flavonoids, alkaloids, phytosterols, natural sugars, vitamins, minerals, and organic acids. In these study Different assay of Antioxidant such as DPPH radical scavenging assay, Scavenging activity of Nitric Oxide, Scavenging activity of Superoxide radical, Scavenging activity of Hydroxyl radical, Reducing Power and chelating activity showed highest activity show in methanol extract of leaves as compared to stem bark. In all extract methanol and petroleum ether show highest antioxidant activity.

Introduction: The products derived from several herbs and plants, being a source of multifunctional curing agents and bioactive compounds, are relatively considered safe for consumption. According to the Food and Agriculture Organization's (FAO) report, about 70–80 % of the world's population, especially in developing countries, relies on herbal medicine to prevent and cure diseases (Ekor 2014), and about 25 % of the synthesized drugs are manufactured from medicinal plants (Pan *et al.* 2013). Increased demand for food to tackle hunger and malnutrition problems has been pertinent in developing countries over the last few decades. In Asian and African countries, the vast majority of the population suffers from malnutrition because of the deficiency of essential nutrients in foods.

Moringa plants that are quite easily found in India because they are easy to grow in areas with tropical climates (Amrullah *et al*., 2019, Roshetko *et al*., 2017). Besides being used as a vegetable, empirically people use moringa and celery for rheumatic drugs, hypertension, xerophthalmia and diabetic. *Moringa* have high tannin, phenol and triterpenoid content, flavonoids, saponins and alkaloids which are very likely to be developed as medicinal plants. Secondary metabolite compounds are closely related to the protection function of the plant itself and can also function for human health. Secondary metabolite compounds are divided into three main groups, namely polyphenol components, including flavonoids and phenols, terpenoids, and alkaloids (R Tiwari *et al*., 2015). Flavonoid are found in all parts of higher plants including leaves, roots, wood, skin, pollen, flowers, fruits, and seeds of varying levels (TY Wang *et al*., 2018) The high and varied secondary metabolite content possessed by

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moringa especially in the leaves, becomes the initial trace to analyze its bioactivity. One of the exciting abilities of bioactivity to be explored from moringa and celery leaves is its activity as an antioxidant. Antioxidants are substances that the body needs to neutralize free radicals and prevent damage caused b y free radicals by complementing the lack of electrolytes that free radicals have and inhibiting the occurrence of chain reactions of free radical formation that can cause oxidative stress (A Prakash *et al*., 2001). Free radicals in the body can attack tissues such as proteins and DNA so that they can trigger diseases such as cancer, premature aging, and other degenerative diseases (Bitzer *et al*., 2019).

Materials and Methods

Collection and Processing of Sample: Plant samples were collected from different parts of Chhattisgarh such as agriculture field, grass land, garden etc. Leaves and stem bark of *Moringa oleifera* were washed with distilled water, chopped into pieces, dried in air and shade, and pulverized very finely in a grinding machine (Prasad and Shekhar,2014).

Extraction of Sample: Extraction are done by two methods Hot Extraction (Soxhlation) and Cold extraction**.**

Phytochemical Analysis of Leaf and Stem Bark Extract

The methods described by Rooplatha and Nair 2013 were used to test the presence of tannins, alkaloids, phytosterols, triterpenoids, flavonoids, saponins, cardiac glycosides, anthraquinone glycosides, carbohydrate, protein, amino acids, fats and fixed oils in all samples, using petroleum ether, chloroform, ethanol and distilled water as solvents. A stock concentration of 1 % (W/ V) of each successive extract obtained using petroleum ether, chloroform, ethanol and water was prepared using the respective solvent. These extracts along with positive and negative controls were tested for the presence of active phytochemicals.

In-vitro Antioxidant Activity

DPPH radical scavenging assay: The effect of extracts on 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical was estimated by using the method described by (Liyana et al., 2006). A solution of DPPH (0.135 mM) was prepared and 1ml of this solution was mixed with 1ml of acetone or aqueous extract (0.02–0.1 mg). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using rutin and BHT as reference drugs. The radical scavenging activity was calculated from the equation:

Percentage of radical scavenging activity = (Abscontrol−Abssample)/ Abscontrol x 100

where; Abscontrol is the absorbance of DPPH radical and acetone; Abssample is the absorbance of DPPH radical and sample extract/standard.

Scavenging activity of Nitric Oxide: The nitric oxide (NO) radical scavenging activity of the aqueous and acetone extracts of *Moringa oleifera* was determined according to the method of Garrat (1964). A volume of 2ml of 10 mM sodium nitroprusside was prepared in 0.5 mM phosphate buffer saline (pH 7.4) and later it was reacted with 0.5ml of plant extract or BHT or rutin at various concentrations (0.02–0.1 mg/mL). The mixture was incubated at 25 °C. After 150 min, 0.5ml of incubation solution was withdrawn and mixed with 0.5ml of Griess reagent ([sulfanilic acid reagent (0.33% prepared in naphthylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, and then absorbance was measured at 540 nm. The amount of nitric oxide radical inhibited by the extract was calculated using the following equation:

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NO radical scavenging activity = {(Abscontrol − Abssample)/(Abscontrol)} x 100
where; Abscontrol is the absorbance of NO radical and methanol; Abssample is absorbance of NO radical and sample extract or standard.

Determination of Superoxide radical scavenging activity: Measurement of superoxide anion scavenging activity of ethanolic and aqueous extracts of *Moringa oleifera* was based on the method described by (Liu *et al.,*1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide anion was generated in 3ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1ml of NBT (50 µM) solution, 1ml of NADH (78 µM) solution and different concentrations (0.1- 1.25 mg/ml) of sample solution. The reaction was started by adding 1ml of PMS-NADH solution (10 µM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and absorbance at 560 nm was recorded against blank samples in a spectrophotometer. Trolox, ascorbic acid and BHA were used as standard samples (0.1- 1.25 mg/ml).

Determination of Hydroxyl radical scavenging activity: The effect of hydroxyl radical was assayed by using the 2-Deoxyribose oxidation method (Chung et al.,1997). 2-Deoxyribose is oxidized by the hydroxyl radical that is formed by the Fenton reaction and degraded to malondialdehyde. The reaction mixture contained 0.45ml of 0.2 M sodium phosphate (pH 7.6), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM FeSO4 -EDTA, 0.15 ml of 10 mM hydrogen peroxide, 0.525 ml of distilled water and 0.075 ml (20-120 µg/ml) of extracts solution in a tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37 °C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% (w/v) trichloroacetic acid and 0.75 ml of 1.0% (w/v) of thiobarbituric acid. The mixture was boiled for 10 min, cooled in ice and then measured at 520 nm. The reaction mixture not containing extracts was used as the control. Trolox, ascorbic acid, BHT and BHA (20-120 µg/ml) were used as standard antioxidants. The scavenging activity on hydroxyl radicals was expressed as: The scavenging activity on hydroxyl radicals = [(A0 – A1) / A0 × 100] Where; A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of extracts.

Determination of Reducing Power: The reducing power of ethanolic and aqueous extracts of *Moringa oleifera* was measured according to the method of Oyaizu.,1986. Various concentrations of extracts (20-140 µg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 7.6) and 2.5 ml potassium ferricyanide [K3 Fe(CN)6] (1%, w/v), and then the mixture was incubated at 50ºC for 30 min. Afterwards, 2.5 ml of trichloroacetic acid (10%, w/v) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper-layer solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%, w/v), and the absorbance was measured at 700 nm. α- tocopherol, BHA and BHT were used as standard antioxidants. Higher absorbance of the reaction mixture indicated greater reducing power

Determination of Chelating activity: The chelating activity of ethanolic and aqueous extracts of *Moringa oleifera* on ferrous ions (Fe2+) was measured according to the method of Decker et al.,1990. Aliquots of 1ml of different concentrations (0.25, 0.50, 0.75 and 1.0 mg/ml) of the extracts were mixed with 3.7ml of deionized water. The mixture was incubated with FeCl2 (2mM, 0.1ml) for 5, 10, 30 and 60 minutes. After incubation the reaction was initiated by addition of ferrozine (5 mM and 0.2ml).for 10 min at room temperature, and then

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the absorbance was measured at 562 nm in a spectrophotometer. A lower absorbance indicates a higher chelating power. The chelating activity of extracts on Fe2+ was compared with that of EDTA at a level of 0.037 mg/ml. Chelating activity was calculated using the following formula:

Chelating activity (%) = [1 – (Absorbance of sample / Absorbance of control) × 100]

Control test was performed without addition of extracts.

Results

Extraction yield: Leaves exhibited higher extraction yield for Methanol (0.87%) and lowest yield for Petroleum ether (0.57%). Stem Bark exhibited higher extraction yield was highest in Methanol (0.73%) and lowest yield for Petroleum ether (0.46%). In Methanol extract both leaves and Stem Bark show high extraction yield and in petroleum ether both show lowest extraction yield. The extraction yields were close for both methods that are soxhletion & maceration.

Table 1:Total % of yield for each extraction procedure

S.No.	*Moringa oleifera*	% yield			
		Chloroform	**Methanol**	**Acetone**	**Petroleum Ether**
1.	**Leaves**	0.67%	0.87%	0.66%	0.57%
2.	**Stem Bark**	0.56%	0.73%	0.52%	0.46%

Table 2: Phytochemical Analysis of *Moringa oleifera* Leaves

Phytochemical Constituents	**Leaf Extracts**			
	Chloroform	Methanol	Acetone	Petroleum Ether
Tannins	-	+	-	-
Alkaloids	+	+	+	+
Phytosterols	+	+	+	+
Triterpenoids	+	+	+	+
Flavonoids	-	+	+	+
Saponins	+	-	-	-
Cardiac glycosides	+	+	-	-
Anthraquinone glycosides	+	+	-	+
carbohydrates	+	+	+	+
proteins	+	+	+	+
amino acids	+	+	+	+
Fats and fixed oils	+	+	+	+

Table 3:Phytochemical Analysis of *Moringa Oleifera* stem bark

Phytochemical Constituents	**Stem Bark Extracts**			
	Chloroform	Methanol	Acetone	Petroleum Ether
Tannins	-	-	-	-
Alkaloids	+	+	+	+
Phytosterols	+	+	+	+

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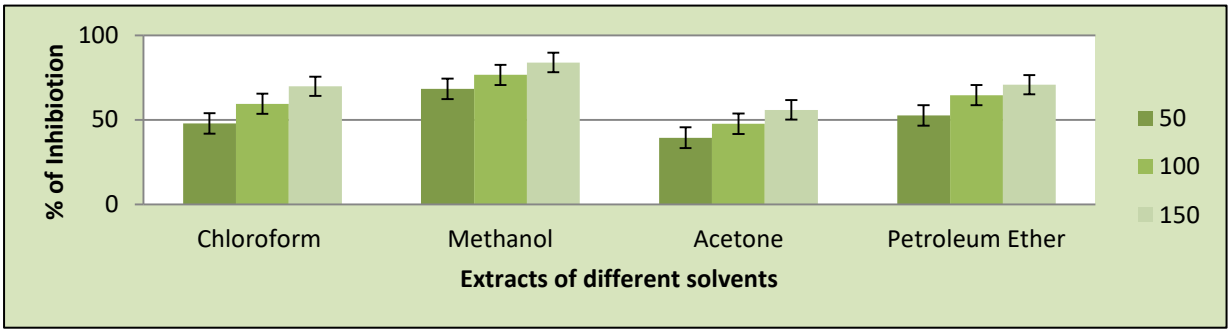
Triterpenoids	+	+	+	+
Flavonoids	-	+	+	+
Saponins	+	-	-	-
Cardiac glycosides	+	+	-	-
Anthraquinone glycosides	+	+	-	+
carbohydrates	+	+	+	+
proteins	+	+	+	+
amino acids	+	+	+	+
Fats and fixed oils	-	+	-	-

In-vitro Antioxidant Activity

DPPH radical scavenging assay of *Moringaoleifera* Leaves: Methanol shows high inhibition in 150µg (83.88%) and Acetone show lowest inhibition in 150µg (39.41%). All four extract Methanol and Petroleum ether show maximum inhibition.

Table 4: DPPH radical scavenging assay of *Moringa oleifera* Leaves

Concentration (µg)	% of inhibition in Different Solvent			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	47.84	68.35	39.41	52.56
100	59.49	76.57	47.67	64.54
150	69.82	83.88	55.85	70.76



Graph1: DPPH radical scavenging assay of *Moringa oleifera* Leaves

DPPH radical scavenging assay of *Moringa Oleifera* Stem Bark:Methanol shows high inhibition in 150µg(73.78%) and Acetone show lowest inhibition in 150µg (44.81%). All four extract Methanol and Petroleum ether show maximum inhibition.

Table5: DPPH radical scavenging assay of *Moringa Oleifera* Stem Bark

Concentration (µg)	% of inhibition in Different Solvent			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	49.44	69.45	44.81	49.57
100	56.45	70.77	50.87	60.64

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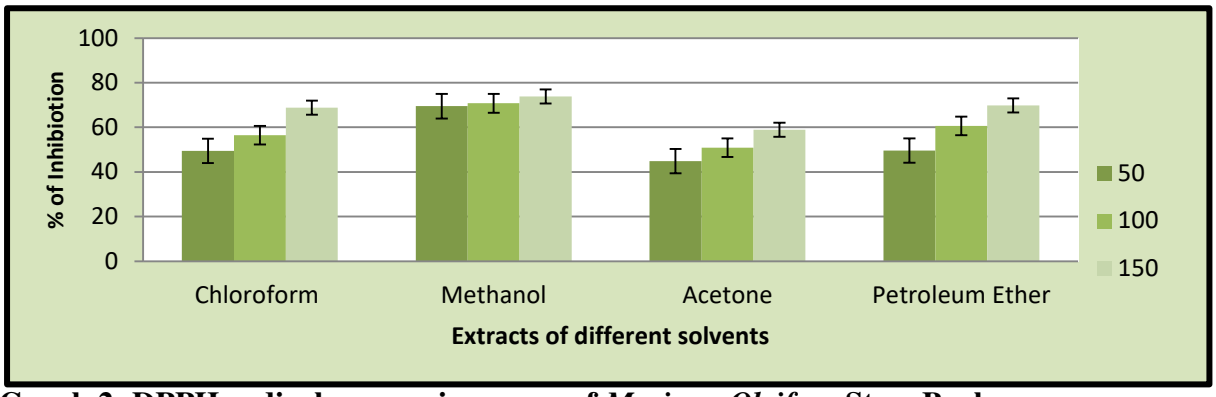
150	**68.82**	**73.78**	**58.85**	**69.76**

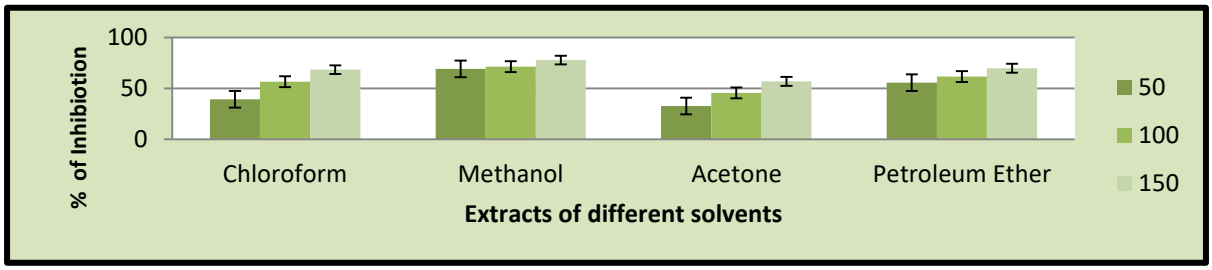


Graph 2: DPPH radical scavenging assay of *Moringa Oleifera* Stem Bark

Scavenging activity of Nitric Oxide: In Leaves Methanol shows high inhibition in 150µg (71.71%) and Acetone show lowest inhibition in 50µg (32.81%). All four extract Methanol and Petroleum ether show maximum inhibition. In Stem Bark Methanol shows high inhibition in 150µg (70.78%) and Acetone show lowest inhibition in 50µg (22.51%). All four extract Methanol and Petroleum ether show maximum inhibition.

Table 6: Scavenging activity of Nitric Oxide for Leaves Extract:

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	39.44	69.06	32.81	55.55
100	56.65	71.17	45.67	61.62
150	68.34	77.78	56.85	69.74



Graph 3: Scavenging activity of Nitric Oxide for Leaves

Table 7: Scavenging activity of Nitric Oxide for Stem Bark Extract:

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether

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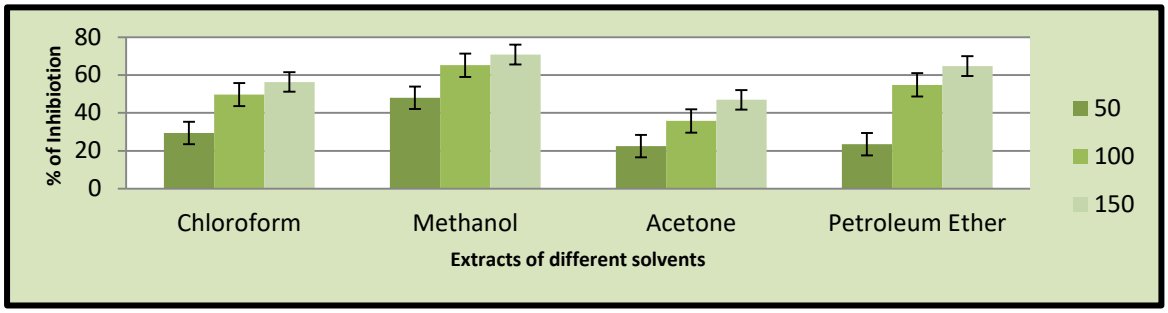
50	29.44	48.06	22.51	23.55
100	49.65	65.17	35.77	54.82
150	56.34	70.78	46.95	64.74

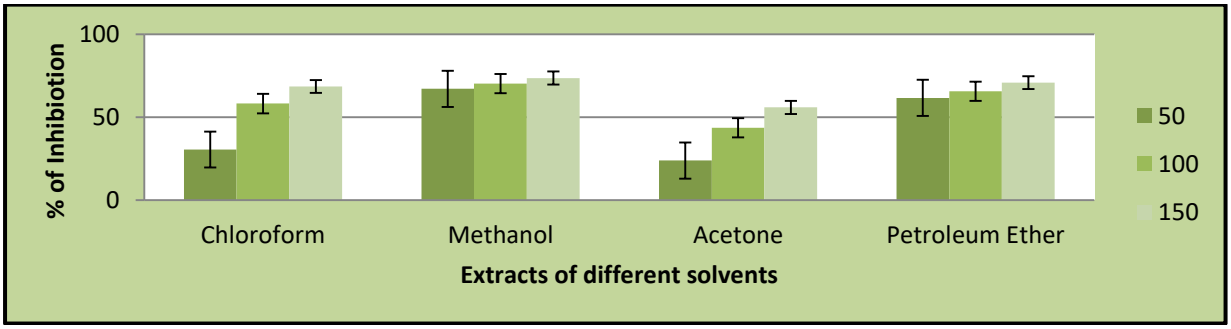


Graph 4:Scavenging activity of Nitric Oxide for Leaves

Determination of Superoxide radical scavenging activity: In Leaves Methanol shows high inhibition in 150µg (73.56%) and Acetone show lowest inhibition in 50µg (23.81%). All four extract Methanol and Petroleum ether show maximum inhibition. In Stem Bark Methanol shows high inhibition in 150µg (70.73%) and Acetone show lowest inhibition in 50µg (23.81%). All four extract Methanol and Petroleum ether show maximum inhibition.

Table 8:Scavenging activity of Superoxide radical scavenging activity for Leaves Extract:

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	30.44	67.06	23.81	61.55
100	58.15	70.18	43.57	65.62
150	68.44	73.56	55.85	70.74



Graph 5: Scavenging activity of Superoxide radical scavenging activity for Leaves Extract:

Table 9:Scavenging activity of Superoxide radical scavenging activity for Stem Bark Extract:

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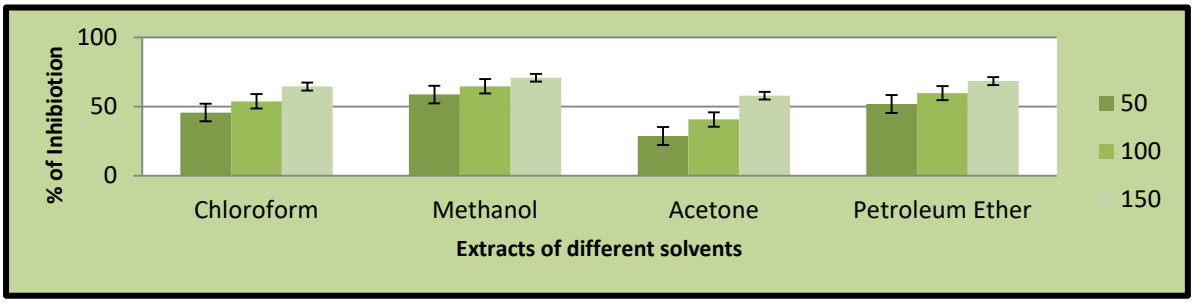
Concentration (µg)	% of inhibition in Different Solvent of Stem Bark extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	45.64	58.66	28.65	51.85
100	53.72	64.61	40.67	59.66
150	64.44	70.73	57.85	68.34

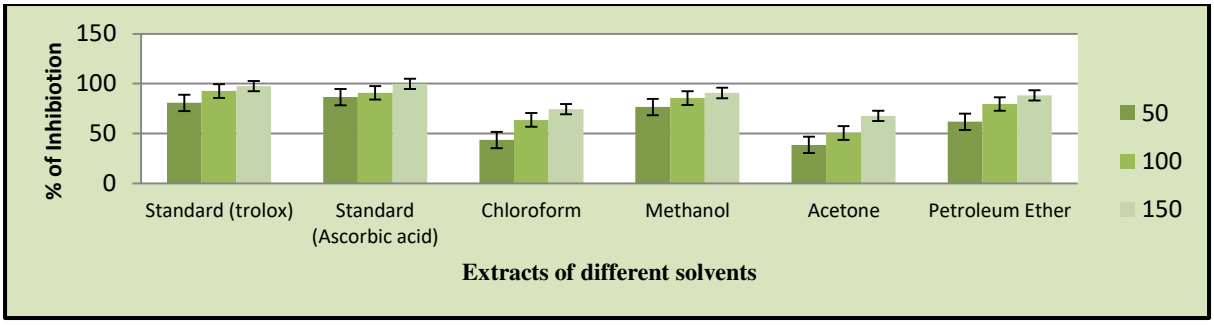


Graph6: Scavenging activity of Superoxide radical scavenging activity for Stem Bark Extract:

Determination of Hydroxyl radical scavenging activity

Table 10: Scavenging activity of Hydroxyl radical for Leaves Extract:

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract					
	Standard (trolox)	Standard (Ascorbic acid)	Chloroform	Methanol	Acetone	Petroleum Ether
50	80.76	86.54	43.64	76.66	38.65	61.85
100	92.68	90.87	63.72	85.61	50.67	79.66
150	97.54	99.76	74.44	90.73	67.85	88.34



Graph 7: Scavenging activity of Hydroxyl radical for Leaves Extract

Table 11: Scavenging activity of Hydroxyl radical for Stem Bark Extract

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract					
	Standard (trolox)	Standard (Ascorbic	Chloroform	Methanol	Acetone	Petroleum Ether

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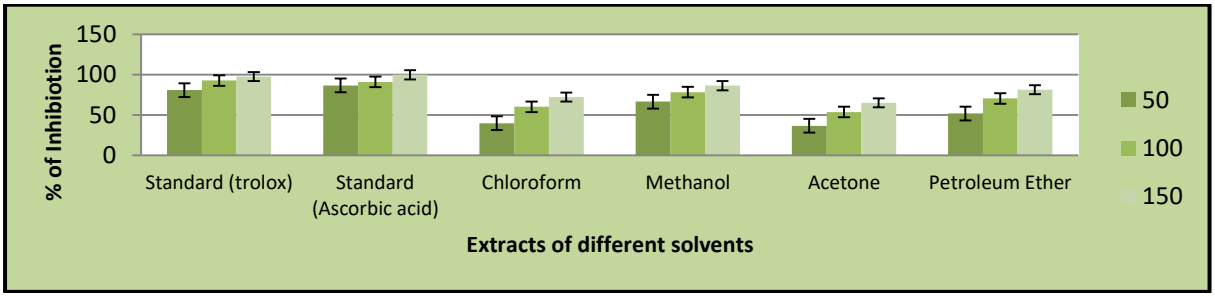
		acid)				
50	80.76	86.54	39.91	66.53	36.75	51.95
100	92.68	90.87	60.12	78.19	53.65	70.56
150	97.54	99.76	72.18	86.28	64.95	81.37

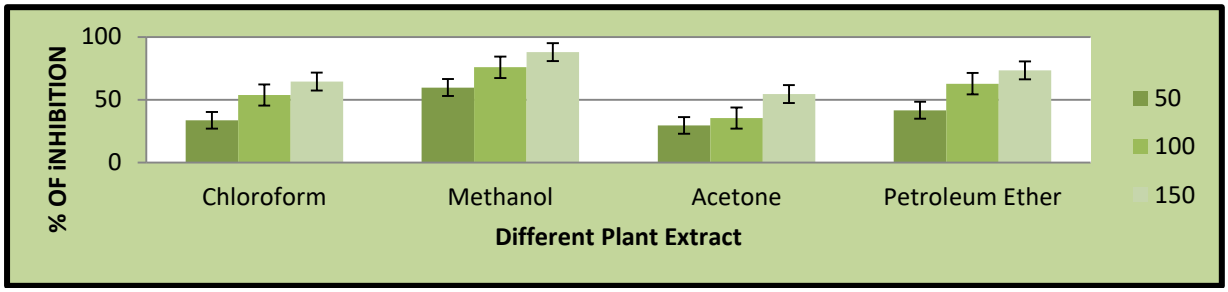


Graph 8: Scavenging activity of Hydroxyl radical for Stem Bark Extract

3.4.5 Determination of Reducing Power

Table 12: Reducing Power of Leaves Extract:

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	33.64	59.71	29.61	41.64
100	53.72	75.83	35.43	62.73
150	64.44	87.93	54.53	73.42



Graph 9: Reducing Power of Leaves Extract:

Table 13: Reducing Power of Stem Bark Extract

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	41.66	61.66	32.65	48.85
100	55.14	70.61	47.67	64.66
150	63.14	80.51	59.85	73.34

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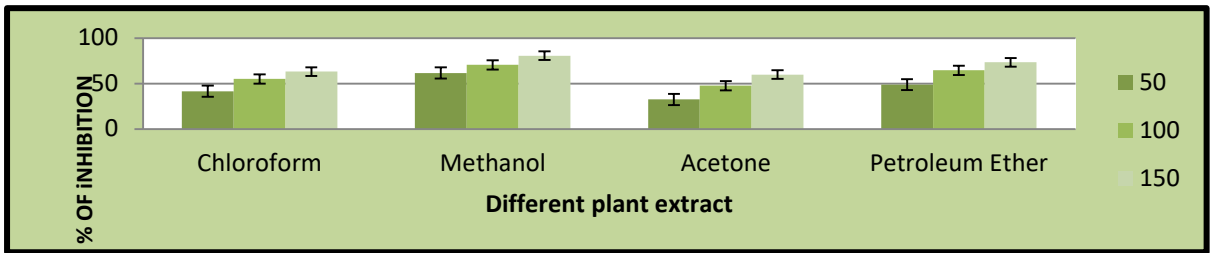
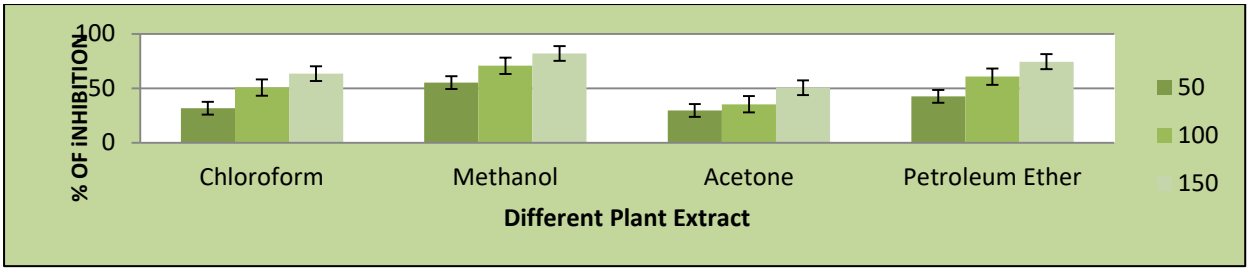


Graph 10: Reducing Power of Stem Bark Extract

Determination of chelating activity

Table 14: chelating activity of Leaves Extract:

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	31.64	55.11	29.61	42.69
100	50.72	70.73	35.43	60.73
150	63.44	81.93	50.53	74.42



Graph 11: chelating activity of Leaves Extract

Table 15: chelating activity of Stem Bark Extract

Concentration (µg)	% of inhibition in Different Solvent of Leaves extract			
	Chloroform	Methanol	Acetone	Petroleum Ether
50	31.26	52.90	27.18	40.85
100	47.64	66.81	42.52	60.42
150	61.57	77.41	53.89	70.47

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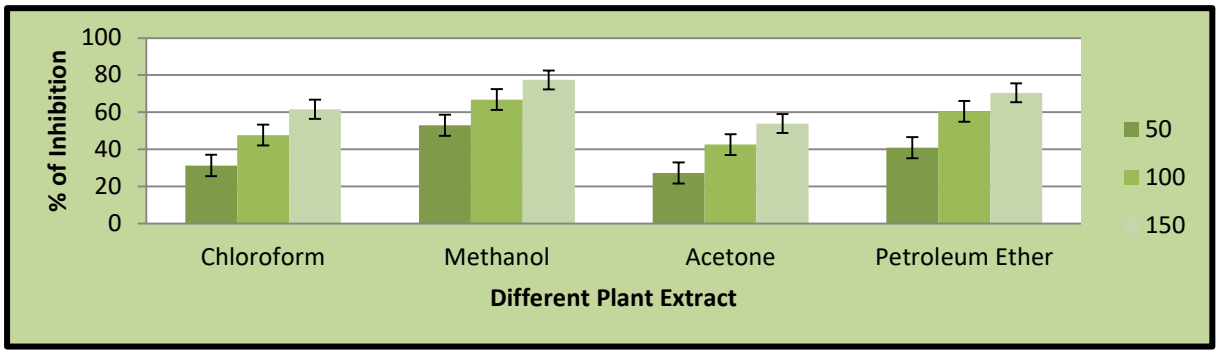

Graph 12: chelating activity of Stem Bark Extract

The experiment shows that *M. Oleifera* has ability of antioxidant properties. In these study leaves and stem bark of *M. Oleifera* has good potential of antioxidant and Phytochemical analysis showed the presence of phytochemical. In all extract of leaves and stem bark of *M. Oleifera* has ability of antioxidant properties but methanol extract leaves are show effective antioxidant properties. Polyphenols and flavonoids found in methanol extracts of *M. Oleifera* are recognized as potent sources of antioxidants. Different assay of Antioxidant such as DPPH radical scavenging assay, Scavenging activity of Nitric Oxide, Scavenging activity of Superoxide radical, Scavenging activity of Hydroxyl radical, Reducing Power and chelating activity showed highest activity show in methanol extract of leaves as compared to stem bark. In all extract methanol and petroleum ether show highest antioxidant activity. Several previous studies also reported that the methanol extract of *M. oleifera* leaves and Stem bark contained polyphenols and had DPPH radical scavenging activity (Kumbhare, *et al.* 2012), (Shahriar, 2012), (Suphachai, 2014). The results reflect that *M. Oleifera* can act as a very good option in the field of medicine based on the antioxidant property of natural products chemistry.

Conclusion

Phytochemical screening of methanol and Petroleum ether extracts of *M. oleifera* and showed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, polyphenols, glycosides, coumarins, and saponins. More phytoconstituents were present in methanol extracts than that of in petroleum ether extracts. Analysis of DPPH free radical scavenging of the extracts showed that both plants are highly potent in terms of antioxidant activity and the extent of antioxidant activity is following the presence of chief phytoconstituents like flavonoids and

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polyphenols present in the plant. Further research is recommended to explore these plants as dietary supplements as a source of antioxidants.

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