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Original Article

EVALUATION OF ANTIOXIDANT POTENTIAL AND PHYTOCHEMICALS OF MORINA LONGIFOLIA

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ABSTRACT

In the present investigation, the antioxidant activities of different polar and non-polar solvent extracts viz. hexane (H), petroleum ether (PE), acetone (AC), choloroform (C), ethanolic (E) and water (W) extracts of whole plant (1 mg/ml) of *Morina longifolia* were determined by standard and routine in vitro antioxidant procedures. The results confirmed that ethanolic extract, acetone extract and choloroform extract of whole plant of *Morina longifolia* exhibited potent antioxidant activity in comparison to that of hexane, petroleum ether and aqueous extracts. The results thus concluded that *Morina longifolia* acts as a potent antioxidant. Further studies are however needed to investigate the potent molecule (s) responsible for antioxidant behaviour in the plant.

Keywords: *Morina Longifolia*, Polar And Non Polar Solvent Extracts, Antioxidant Activity, Potent Molecules.

INTRODUCTION

India is one of the nations blessed with a rich heritage of traditional medical systems and rich biodiversity to complement the herbal needs of the treatment administered by these traditional medical systems. The recognized Indian Systems of Medicine are Ayurveda, Siddha and Unani, which use herbs and minerals in the formulations. The Health advantages of diets rich in antioxidant plant compounds include lowering the risk of cardiovascular disease, certain cancers and the natural degeneration of the body associated with the ageing process. Free radicals are unstable molecules formed when the body uses oxygen for energy. The instability of these molecules can damage tissues, alter DNA and change cell structure. Ultimately, free radicals start a chain reaction resulting in the reproduction of even more free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage, free radicals cause to the body. The role antioxidants have in free radical stabilization involves the antioxidants donating one of their own electrons to the free radical. This electron donation is done without the antioxidant becoming unstable or damaging to the body. This remarkable action stabilizes the free radicals as quickly as they are produced in the human body. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavanoids [1] which prevents free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease [2]. The search for newer natural antioxidants and antimicrobials especially of plant origin has ever since increased. In this study, the Traditional solvent extraction (TSE) methods were used for extraction of antioxidants. The results can determine the natural antioxidants available in the plant parts having solubility in the specific solvent. Also, the extraction methods will emphasize on using the specific solvent (hexane, petroleum ether, chloroform, acetone, ethanol and water) for extracting antioxidants and polyphenolics. *Morina* species showed that, a new aromatic glycoside characterized as 2,6-dihydroxy-5-methoxy-(3-Cglucopyranosyl) benzoic acid was isolated along with four known compounds from the aerial parts of *Morina longifolia* [3]. A novel acylated flavonol glycoside, quercetin-3-O-[2'''-O-(E)-caffeoyl]-α-L-arabinopyranosyl-(1,6)-β-D-galactopyranoside, was isolated from whole plant of *Morina nepalensis* [4]. Two novel tetrahydropyran sesquineolignans with a new carbon skeleton, named morinols A-B and other ten novel neolignans, named morinols C-L along with two known lignans, pinoresinol and lariciresinol, have been isolated from the roots of Chinese medicinal herb, *Morina chinensis* [5]. The higher percentage of the most of bioactive constituents has been found in the essential oil collected in the month of January [6]. Major constituents in leaf oil of *Morina longifolia* were germacrene D (10.75 %) pinene (4.84 %), bicyclogermacrene (4.26 %), cadinol (4.26 %), (*E*)-citronellyl tiglate (4.20 %), phellandrene (3.24 %) [7]. Compared to the reference antioxidant compounds, the oil of *Morina longifolia* showed antioxidant capacity comparable to BHT and α-tocopherol and higher than BHA in Oxygen Radical Absorbance Capacity (ORAC) assay [8]. The root paste has been applied externally on wounds and the aroma of the flowers has been used for unconsciousness in Indian traditional medicine [9]. Antimicrobial potential of some plants of Uttarakhand were investigated [10-12]. This study may provide insight for future extraction solvents and natural potent antioxidants which can be used as dietary supplements.

MATERIALS AND METHODS

Plant Materials

The plant material was collected from Garhwal region of Uttarakhand, India. The plant material was identified from Botanical Survey of India, Dehradun. Voucher specimen of the plant was stored in the Department of herbarium for future reference. The plant material was dried in the shade in an open air for 5-10 days so that fine powder can be formed.

Preparation of Plant extracts

Plant parts were separated, washed with distilled water, dried under shade and pulverized. The plant extracts were prepared according to the method prescribed with little modifications [13]. Briefly 20 g portions of the powdered plant material was soaked separately in different solvents i.e. petroleum ether, hexane, chloroform, acetone, ethanol and distilled water on the basis of increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England). The filtrates obtained were concentrated in vacuom using water bath at 30 ^{0}C .

Determination of *In vitro* Antioxidant activity

Determination of Total Phenolic Content (TPC)

The Total Phenolic Content of each extract obtained from each of the plant extract was determined [14] and the phenolic content was

expressed as µg/g Gallic acid equivalents. In brief a 100 µl aliquot of the sample was added to 2 ml of 0.2% (w/v) Na_2CO_3 solution. After two minutes of incubation, 100 µl of 500ml/l Follin-Ciocalteu reagent added and the mixture was then allowed to stand for 30 minutes at 25^{0}C. The absorbance was measured at 750 nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The Total Phenolic Content (TPC) was determined using the standard Gallic acid calibration curve and was expressed as µg/g Gallic acid equivalents.

Determination of Antioxidant Activity by DPPH Radical Scavenging Method

The extract solution for the DPPH test [15] was prepared by re-dissolving 0.2 g of each of the dried extract in 10 ml of the specific solvent in which the extract was prepared. The concentration of DPPH solution was 0.025 g in 1000 ml of methanol. Two ml of the DPPH solution was mixed with 40 µl of each of the plant extract solution and was transferred to a cuvette. The reaction solution was monitored at 515 nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible Systronics spectrophotometer. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation: Inhibition%= (Abst=0 min---Abst=30 min)/ Abst=0 min ×100 Where Abst=0 min was the absorbance of DPPH at zero time and Abst=30 min was the absorbance of DPPH after 30 minutes of incubation. Ascorbic acid (0.5 mM) dissolved in methanol was used as a standard to convert the inhibition capability of plant extract solution to the Ascorbic acid equivalent. IC_{50} is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Superoxide Anion Radical Scavenging Activity

Superoxide Anion Radical scavenging Activity was measured according to the method [16] with some modifications. The different plant extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using Systronics UV-VIS double beam spectrophotometer. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control.

The Superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{Ao - As}{Ao} \times 100$$

Phytochemical screening of the extracts

The portions of the dried extracts were subjected to the phytochemical screening using the method adopted [17,18]. Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars and cardiac glycosides.

Test for alkaloids

The 0.5 g of the plant extracts were dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent Turbidity or precipitation was taken as indicator for the presence of alkaloids.

Test for Tannins

About 0.5 g of the sample were dissolved in 10 ml of boiling water and was filtered. Few ml of 6% $FeCl_3$ was added to the filtrate. Deep green colour appeared confirmed the presence of Tannins.

Test for Flavanoids

About 0.2 g of the extracts were dissolved in methanol and heated for some time. A chip of Mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was Indicator of the flavanoids.

Test for Saponin

About 0.5 g of the plant extracts were stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

Test for Steroids

Salkowaski method was adopted for the detection of steroids. About 0.5 g of extracts were dissolved in 3 ml of chloroform and filtered. To the filtrate concentrated H_2SO_4 was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring.

Test for Cardiac glycoside

About 0.5 g of the extracts were dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% $Fecl_3$. This was under laid with concentrated H_2SO_4. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

Test for reducing Sugars

1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extracts. The mixtures were heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

RESULTS AND DISCUSSION

Antioxidant activity

In-vitro antioxidant activity was determined by DPPH radical scavenging method and Superoxide anion radical scavenging assay. The results confirmed that ethanolic extract, acetone extract and choloroform extract of whole plant of *Morina longifolia* exhibited potent antioxidant activity in comparison to that of hexane, petroleum ether and aqueous extracts. Amongst all the extacts, ethanolic and acetone extract showed potent antioxidant activity as determined by the procedures. TPC in ethanol extracts was found to be 224 µg/ml followed by acetone extracts having 215 µg/g gallic acid equivalents. IC_{50} value of ethanol extracts was found to be 17.10 µg/ml followed by acetone extracts viz. 25.10 µg/ml in DPPH radical scavenging method. It was found that minimum is the value of IC50, maximum is the antioxidant activity. In Superoxide anion radical scavenging method ethanol extracts showed 72 % inhibition of superoxide followed by acetone extracts having 60 % inhibition. Ascorbic acid was used as the standard antioxidant having IC_{50} value, 78.17 µg/ml in DPPH radical scavenging method and causes 87.80 % inhibition of superoxide. The results are shown in **Table 1**, **2** and **3**; **Figure 1, 2** and **3.** The results of all the three procedures are totally correlated to each other and confirm the use of plant as natural antioxidant. Presence of polyphenolics in the extract confirm their utility as potent antioxidant agent as revealed by the experimental results. Traditional Solvent Extraction (TSE) method was found to be efficient for extraction of antioxidants [19]. If plant extracts are to be used for medicinal purposes, the issues of safety and toxicity should be monitored [20].

Table 1: TPC (µg/g gallic acid equivalents) of solvent extracts of *Morina longifolia*

***Morina longifolia* (Solvent Extracts)**	**TPC (µg/g gallic acid equivalents)**
Ethanol extract (E)	224
Acetone extract (AC)	215
Water extract (W)	150
Hexane extract (H)	135
Chloroform extract (C)	178
Petroleum ether extract (PE)	120

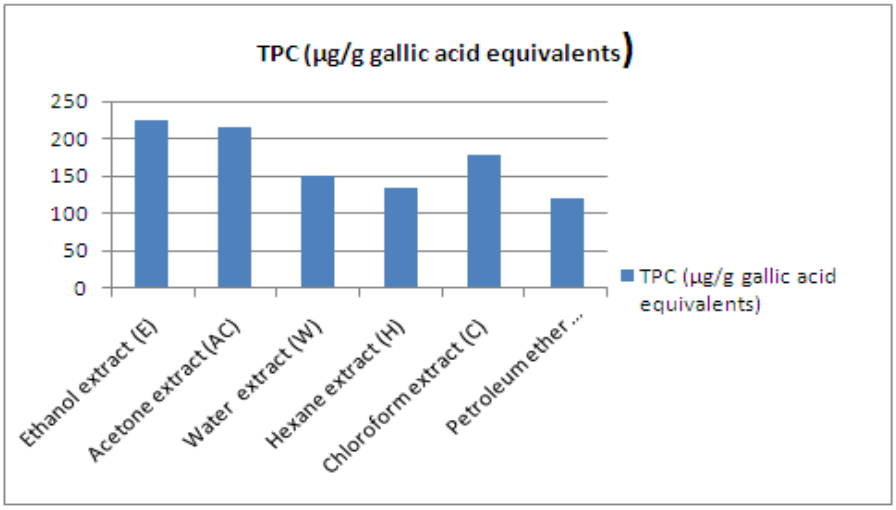

Fig. 1: TPC (µg/g gallic acid equivalents) of solvent extracts of *Morina longifolia.*

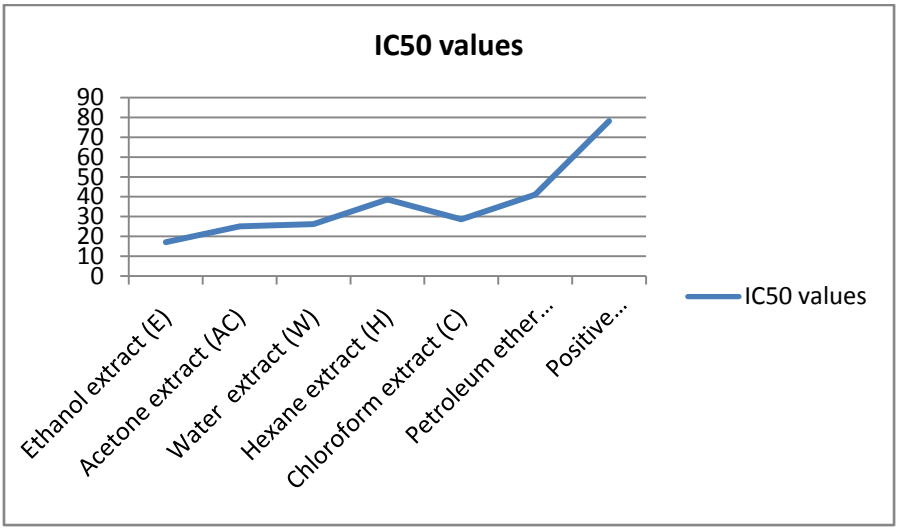

Fig. 2: IC50 values of solvent extracts of *Morina longifolia* as determined by DPPH assay.

Table 2: IC50 values of solvent extracts of *Morina longifolia* as determined by DPPH assay

***Morina longifolia* (Solvent Extracts)/ Positive Control**	**IC50 values**
Ethanol extract (E)	17.10
Acetone extract (AC)	25.10
Water extract (W)	26.20
Hexane extract (H)	38.65
Chloroform extract (C)	28.67
Petroleum ether extract (PE)	41.05
Positive Control, Ascorbic acid	78.17

Table 3: Percent inhibition of superoxide free radicals of solvent extracts of *Morina longifolia* as determined by Superoxide anion radical scavenging activity

***Morina longifolia* (Solvent Extracts)/ Positive Control**	**Percent inhibition of Superoxide free radicals**
Ethanol extract (E)	72.0
Acetone extract (AC)	60.0
Water extract (W)	43.50
Hexane extract (H)	35.50
Chloroform extract (C)	45.0
Petroleum ether extract (PE)	32.0
Positive Control, Ascorbic acid	87.80

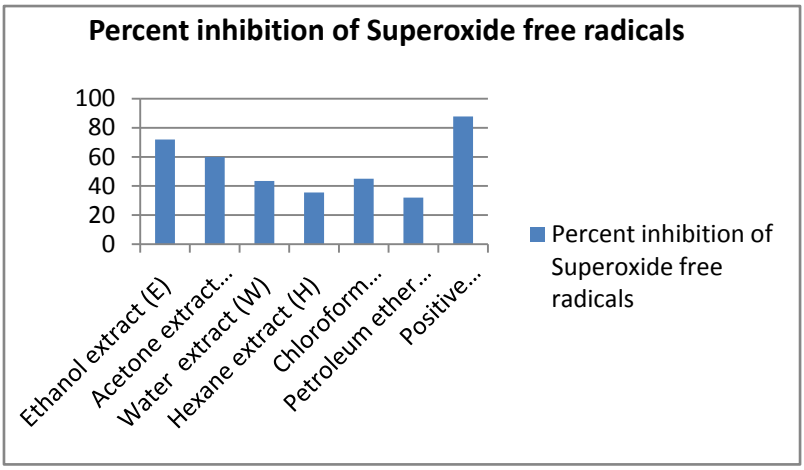

Fig. 3: Percent inhibition of superoxide free radicals of solvent extracts of *Morina longifolia* as determined by Superoxide anion radical scavenging activity.

Table 4: Phytochemical screening of solvent extracts of *Morina longifolia.*

Morina longifolia (Solvent Extracts)	Phytochemicals						
	Alkaloids	Tannins	Flavanoids	Saponin	Steroids	Cardiac glycosides	Reducing Sugars
Ethanol extract (E)	+	+	+	+	+	+	+
Acetone extract (AC)	+	+	+	+	+	+	+
Water extract (W)	+	+	+	+	+	+	+
Hexane extract (H)	_	_	_	+	+	_	_
Chloroform extract (C)	+	+	+	+	+	+	+
Petroleum ether extract (PE)		_	_	+	+	_	_

*+, presence; -, absence

Phytochemical Screening

Different conventional methods were followed to determine qualitatively the presence of phytochemical constituents present in the potent extract. Only steroids and saponin were observed in hexane and petroleum ether extracts while all other extracts possessed all the phytochemicals. The results are indicated in **Table 4.** The study thus highlighted the importance of pharmacological importance and scientific investigation of plants from North West Himalaya Garhwal region through forward bio-prospection to uncover bioactive phytochemicals of interest and thus validates traditional medicine.

CONCLUSION

Amongst all the extracts, ethanolic and acetone extracts showed potent antioxidant activity as determined by the different procedures. TPC in ethanol extract was found to be 224 µg/ml followed by acetone extracts having 215 µg/g gallic acid equivalents. IC50 value of etanol extract was found to be 17.10 µg/ml followed by acetone extracts viz. 25.10 µg/ml in DPPH radical scavenging method. In Superoxide anion radical scavenging method etanol extracts showed 72 % inhibition of superoxide followed by acetone extracts having 60 % inhibition. Anti oxidation activity of different plant extracts lead to the formulation of some antioxidants. Only steroids and saponin were observed in hexane and petroleum ether extracts while all other extracts possessed all the phytochemicals. Presence of these compounds in the different extracts confirmed their utility as potent antioxidant agent as revealed by the experimental results. Further work is needed for the isolation and characterization of the active compounds which are responsible for anti oxidation activity.

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REFERENCES

1. Diplock AT, Charleux JL, Crozier WG, Kok FJ, Rice EC, Roberfroid M. Correlation between antioxidants and polyphenolic compounds in fruits and vegetables. Br. J. Nutr 1998; 80:577.
2. Hu FB. Role of antioxidant in preventing cardiovascular diseases. Curr Opin Lipidol 3 2000;13
3. Bodakhe SH, Ram A, Pandey DP. A new aromatic glycoside from Morina longifolia Wall. Asian Journal of Chemistry 2010;22 SRC - GoogleScholar:89-93.
4. Tang R, Xie H, Liu X, Wang D, Yang C. A novel acylated flavonol glycoside from Morina nepalensis var. alba. Fitoterapia 2002; 73: 1.
5. Su BN, Takaishi Y, Kusumi T. Twelve Novel Sesquineolignans and Neolignans Morinols A-L, With a New Carbon Skeleton isolated from Marina chinensis Tetrahedron 1999; 55: 14571-14586.
6. Kothiyal SK, Semwal DK, Badoni R. Seasonal variation in the essential oil composition from Morina longifoli. International Journal of Essential Oil Therapeutics 2009;3 SRC - GoogleScholar:171-4.
7. Joshi RK, Mathela CS. Composition of Morina longifolia from Himalayan region of Uttrakhand, Asian J. Res Pharm. Sci. 2013; 3: 12-14.
8. Kumar M, Sharma S, Vasudeva N. In vivo assessment of antihyperglycemic and antioxidant activity from oil of seeds of brassica nigra in streptozotocin induced diabetic rats. Advanced pharmaceutical bulletin 2013;3(2):359-65.
9. Gaur RD, Trans I. Flora of District Garhwal North West Himalaya (With ethno botanical notes). 1st ed1999.
10. Yousuf S, Bachheti RK, Joshi A, Bhat MUD. In vitro screening of different extracts of Morina longifolia on pathogenic microorganisms. International Journal of Pharmacy and Pharmaceutical Sciences 2011;3
11. Yousuf S, Bachheti RK, Joshi A. Screening of extracts of Valeriana hardwickii for their antibacterial activity. Int J Pharm Tech Res 2013;5Yousuf S, Bachheti RK, Joshi A. Comparative analysis of in

vitro antibacterial activity of extracts of Viola patrinii on pathogenic microorganisms. Int J Res Pharm Sci 2012;3

12. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. Journal of ethnopharmacology 1993;39(3):171-4.
13. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. American Journal of Enology and Viticulture 144 1965;16 SRC - GoogleScholar.
14. Fargare T. In vitro antioxidant activity of plant extracts by DPPH assay. Eur Poly J 1995;31 - Duan X, Wu G, Jiang Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules (Basel, Switzerland) 2007;12(4):759-71.
15. Trease GE, Evans WC, Brown E. Pharmacogonasy, 14th. BMJ (Clinical research ed.) 1989.
16. Harborne DJ, Worrell J. Accident and emergency in London. Good primary care reduces workload. BMJ (Clinical research ed.) 1993;306(6894):1752.
17. Mathur A, Mathur D, Prasad G, Dua VK, Asian J. Microwave Solvent Extraction (MSE) as an effective technique Against Traditional Solvent Extraction (TSE) for Screening Different Plant extracts for Antioxidant Activity. Biochemical and Pharmaceutical Res 2011;2(1 SRC - GoogleScholar):410-8.
18. Doppalapudi S. Evaluation of antibacterial activity of seed extracts of vigna unguiculata. International Journal of Pharmacy and Pharmaceutical Sciences 2014;6(1 SRC - GoogleScholar):75-7.