Phytochemical screening, antioxidant and *in vivo* neuropharmacological effect of *Monotheca buxifolia* (Falc.) barks extract

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Abstract: Owing to its pharmacological versatility, the current study focuses the evaluation of *Monotheca buxifolia* (*M. buxifolia*) bark crude extract and its fractions for phytochemical and pharmacological analysis. Phytochemical investigation of bark extract was carried out through GC-MS, LC-MS and FT-IR. ICP-OES was used for analyzing essential metals in bark extract. Plant samples were further investigated for their *in vitro* antioxidant and *in vivo* neuropharmacological activities in mice. Phytochemical analysis of bark extract revealed the presence of various active constituents such as serotonin, α-tocopherol, 3-deoxyestradiol, ascorbyl palmitate and cirsimaritin. Metal analysis showed presence of various metals in diverse concentration. *M. buxifolia* bark extract and its chloroform fraction showed significant antioxidant activity against DPPH (89.55 ±1.29; 84.80±1.66%), superoxide (82.10 ±1.86; 80.0±1.0%), H_2O_2 (80.55±2.0; 78.10±2.26%) at 500µg/mL concentration. Similarly, bark extract and its chloroform fraction demonstrated antidepressant activity in mice and improve generalized locomotive behavior. The effective use of *M. buxifolia* in treatment and management of depression and free radicals based disorders can be safely concluded from the results of present study.

Keyword: *M. buxifolia*, GC-MS, FT-IR, antioxidant, free radical scavenging, neuropharmacology.

INTRODUCTION

Plants being an alternative source of drugs, about 80% of the world population used them for cure of various diseases (Fatima, Baig, Hasan, & Ahmed, 2018). Mood disorders are the leading cause of psychological problems, further they can be increased due to stress and oxidation process. Drugs for such disorders has 60% success rate and significant number of individuals do not well respond to currently marketed drugs (Wong and Licinio, 2001).

Monotheca buxifolia (Falc.) of Sapotaceae family grows in Northern Pakistan and Afghanistan and its fruit is locally called Gurgura. Earlier the plant fruits have been reported to contain appreciable levels of phenol and flavonoid contents. The plant fruit was also previously evaluated for its reducing power and free radical scavenging capability using DPPH, ABTS, phosphomolybdate, superoxide, hydroxyl radicals and hydrogenperoxide free radicals (Jan, Khan, Rashid, & Bokhari, 2013).

The leaves of *M. buxifolia* also exhibited a strong notable antioxidant ability (Rehman, Khan, Farid, Kamal and Aslam, 2013). The plant also has considerable antimicrobial Hazrat, Nisar and Zaman (2013) antipyretic, anti-inflammatory Ullah *et al*., (2016) and renal protective effect (Khan and Jan, 2016). Locally, the plant is used to cure digestive and laxative disorders (Marwat *et al*., 2011). Previously plants containing antioxidant compounds were reported for neuroprotective effects (Halliwell, 2001, Olanow, 1993). Due to lack of research on barks of *M. buxifolia*, the present study has been designed to evaluate its phytochemical, antioxidant and neuropharmacological effects. The general locomotor behavior in mice will also be observed in this study.

MATERIALS AND METHOD

Chemicals

1,1-diphenyl-2-picryl-hydrazil (DPPH) were procured from Roche Diagnostics, Mannheim (Germany), (Ascorbic and all solvents) Sigma Aldrich (USA). potassium hexacyanoferrate [K3Fe (CN)6], F-C reagent, sodium carbonate (Na_2CO_3), butylatedhydroxyanisol (BHA), Ferric chloride, trichloroacetic acid (TCA) were purchased from MP Biomedicals (France). H_2SO_4 and HCl were all obtained from Fischer Scientific (USA), *n*-Hexane Merck (Germany), ethyl acetate, and chloroform Riedel-de Haen (Germany). Diazepam and imipramine were arranged from Roche, and Novartis Pharma (Pak) Ltd Pakistan.

Plant sample collection

The barks of *M. buxifolia* were collected in June 2016 from the hilly region of Malakand, KPK, Pakistan.

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Initially, the plant was identified via its local name Gurgura followed by its authentication by Dr. Mansoor Ahmad, (Research Institute of Pharmaceutical Sciences), University of Karachi, Pakistan. A voucher specimens no. (SB/01/15-MB) was submitted in the (PIPS), University of Karachi, Pakistan.

Extraction and fractionation

Plant barks were rinsed with tap water and subjected to drying under shade. Dried bark (6kg) was pulverized and macerated in analytical grade methanol for 15 days. Furthermore, the solvent was filtered using Whatmann filter paper and concentrated using rotary (B-490, Buchi) evaporator at 45°C, giving greenish crude methanolic extract (CME, 118.10gm, 1.968%). For further fascination in distilled water (100gm) CME was suspended and then was treated with different solvents i.e. *n*-hexane, ethyl acetate and chloroform respectively using separating funnel. It gave 25.0gm (25%), 18.5gm (18.5%) and 21.3gm (21.3%) *n*Hexane (NHF), ethyl acetate (EAF) and chloroform (CHF) fractions respectively. The remaining portion was aqueous fraction (AQF, 35gm, 35%). Prior experimental use, aliquots were protected from light and kept at 4°C (Jovanovic, Kitic, Palic, Stojanovic, & Ristic, 2005).

Gas-chromatography analysis

CME was examined using gas chromatograph (Agilent USB-393752, USA) with HHP-5MS (5%) phenylmethylsiloxane capillary column (30 m×0.25 mm × 0.25μm) assembled with an FID detector. Initially the oven temperature was maintained at, 70°C, for 60 sec. Which was then gradually increased at the rate of 6°C per min up to 180°C for 5 min. In the next 20 minutes' temperature was increased at the rate of 5°C/min up to 280°C for 20 min. The temperature of the injector and detector temperatures were kept at 220 and 290°C, respectively. Helium was supplied with flow rate of 1 ml/min as carrier. The sample was diluted up to 1/1000 in *n*-pentane, and 1.0μl *v/v* of sample was injected physically into the split-less mode.

Gas-chromatography mass spectrometry (GC/MS) analysis

GC/MS investigation of CME was processed using a gas-chromatograph with a HHP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25mm×0.25μm) outfitted with mass selective detector (Agilent HP-5973, USA) in the electron impact mode (Ionization energy: 70 eV) working under the same experimental conditions as described for GC (Ibrahim, Kainulainen, Aflatuni, & Tiilikkala, 2012).

Components identification

The CME principal ingredients were identified by correlating their retention times with the compound reported in authentic literature. Furthermore, recognition was carried out through the spectral data acquired from the Wiley and NIST libraries. The mass spectra fragments were also correlated with available data in the literature (Stein *et al*., 2002; Adams, 2007).

Isolation and purification

A normal phase silica gel column was loaded with 10g CHF, the fraction was eluted by gradient mobile phase, *i.e.,* Hex: EtOAc (100:0-0:100). Finally, two pure compounds, *i.e.,* stigmasterol (20mg) and Cirsimaritin (12.3mg).

FT-IR analysis

FT-IR spectra of crude methanolic extract of *M. buxifolia* was measured using deuterated tri-glycine SO_4 (DTGS) coupled detector spectrometer (Thermo Nicolet FT-IR Nexus) in the mid-IR region i.e. 4000-400cm^{-1} at resolution 4cm^{-1} with 16 scans as reported by (Oliveira *et al*., 2016).

Multiple metals quantification

CME was subjected to multiple metals i.e. Cd, Pb, Na, K, Zn, Mn, Fe, Mg, Ca and Si on Inductively-coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Optima 2000). The method was performed as describe by Wheal, Fowles and Palmer, (2011) with slight modification. About 1gm of CME was heated on a hot plate at 250°C for 10-15min to evaporate methanol fallowed by an ashing process in a furnace at 550°C for 6-7hours. In the ash we used concentrated H_2SO_4 and HCl instead $HNO_3/HClO_4$ was added to digest the residue (ash) and then 15ml deionized water was added to dissolve the ash and then filtered with 0.45 μm filter paper. Blank was prepared similarly. On next day sample and blank was run on ICP-OES to get the spectra.

Antioxidant activity assay

DPPH radical scavenging potential

The DPPH scavenging potential was carried out as reported by (Blois, 1958). Briefly 2.4mg of DPPH was dissolved in 100 mL methanol and stored in dark at 20°C until need. Then 2mL of various concentrations (100-500µg/mL) of bark extract were mixed with 1mL of DPPH stock solution and incubated for 15 minutes in dark. Samples were read at 517nm. Whereas ascorbic acid was used as a standard. This procedure was performed in triplicate and DPPH scavenging capability was calculated as follows;

$$\text{Percentage inhibition (\%)} = \left[\frac{\text{(Absorbance of control-Absorbance of sample)}}{\text{Absorbance of control}}\right] \times 100$$

Superoxide free radical scavenging potential

The super oxide scavenging potential assay was performed, as reported (Beauchamp & Fridovich, 1971). The reaction solution comprised of (500mL of 50mM PO_4 buffer (pH 7.6), 300mL of 50mM riboflavin, 250mL of mM phospho-methozine SO_4, and 100mL of 0.5mM nitroblue tetrazolium. About 1mL of extract samples with 1 mL of reaction mixture was determined at 560nm. The

standard ascorbic acid was also performed using similar procedure and performed in triplicate. The percent inhibition of free radicals was calculated as:

Percentage inhibition (%)= (1-sample absorbance/control absorbance) x100

Hydrogenperoxide scavenging activity

This assay was performed in accordance with the procedure of (Ruch, Cheng, & Klaunig, 1989). The reaction solution was comprising H_2O_2 (2 mM in 50 mM phos-phate buffer (pH 7.4)), followed by plant extract sample preparation of (0.1 mL) in test tube and the previously prepared 50 mM phosphate buffer (pH 7.4) was added to the aliquots with volume make up to volume 0.4 mL. Moreover, 0.6 mL of H_2O_2 was added to the mixture solution, followed by absorbance execution at 560 nm. This procedure was repeated for standard ascorbic acid, and performed in triplicate. The potential of H_2O_2 free radical scavenging was calculated using below equation:

Percentage inhibition (%)= (1-sample absorbance/control absorbance) x100

Neuropharmacological activity

Animals

Male and female Albino mice (20–25 g) were used for all neuropharmacological activities. Animals were procured from Dow University of Health Sciences. All the experimental animals were maintained according the standard laboratory settings i.e. (25°C and 12/12 hrs. dark / light cycles) and served with adequate feed and water and libitum.

Neuro-pharmacological potential of CME bark and its fractions were carried out by open field, traction, head dip, rearing, and swimming induced depression test. All experiments were carried out in a placid and relaxed setting. Animals were distributed in such a way that, each test was comprised on 6 animals, under 5 groups i.e. Group (A=control), Group (B, C and D drug concentrations were 100, 300 and 500mg/kg) dose of CME and its subsequent fractions. Animals in Group E were administered with standard drug through oral route. The animals in negative-control group A were supplemented with similar volume of normal saline.

Open field activity

This method was implies as defined by (Gupta, Dandiya, & Gupta, 1971). In this test, the animals were grouped as discussed above. CME and its subsequent fractions of *M. buxifolia* at doses of 100, 300 and 500mg/kg, body weight was administered to test groups through oral route. On the other hand, the control group was served with vehicle 1% tween 80 in water. The apparatus consisted of a (76 × 76 cm) square area and 42 cm high with opaque walls. The bottom of the chamber was calibrated into a series of 25 equal squares. Once the animals were administered with oral doses of all the extract. The animals were placed in the middle of chamber and the squares crossed by the animals were counted for 15 and 30 min

Rearing test

For this activity a 1000-mL glass beaker was used and placed on white surface. Prior activity the animals of group A were served with normal saline, while, group B, C, D were served with *M. buxifolia* crude barks extract and its subsequent fraction. Group E receive diazepam 2mg/kg. The upward mobility (an upright position of mice in beaker) were recorded for 30 minutes as reported by (Yadav, Kawale, & Nade, 2008).

Hole cross test

A 40x40cm wooded board box with 16 evenly spaced hole was used for this experiment. The similar group distribution i.e. Group A, B, C, D, and E were used having 6 animals in each group. Group A received normal saline, Group B, C, and D received CME/fractions, while to Group E administered diazepam 2 mg/kg as a standard. After 30 minutes the negative-control and extract-treated mice were placed one by one in box. During a period of 10 minutes the total number of head dip by the animals through the holes were counted (Kennett, Dickinson, & Curzon, 1985).

Traction test

For traction time the apparatus was designed such that, the animals were allowed to travel on metal bar of 1 meter in measurement. The animals were learned to walk on the iron rod prior experiment. The animals were divided in the same pattern of groups as described in hole cross test, followed by administration of normal saline, CME/fractions, and diazepam 2 mg/kg. The stimulatory or sedative effect of drug on mice will either enhance or diminish the time required by animals to travel the rod (Sanchez-Mateo, Prado and Rabanal, 2002).

Forced swimming test

Forced swimming test (FST) was conducted in a translucent glass container as reported by Porsolt *et al*., (1977) with slight modification, that we use rectangle container (50×25cm) length and width having 30 cm height. All animals were grouped as previously described. Prior experiment all mice were allowed to acclimate in a water bath (10 cm height) for 6 minutes at a temperature of (24-25°C). During 6-min test session of the period of immobility (sec) was measured. The time duration was noticed as “immobile” if movement with all limbs in a paddling manor is not observed. Otherwise, movement like keeping head above water level is necessary for survival.

STATISTICAL ANALYSIS

Results were presented as mean ± SD. Significance of results were calculated by ANOVA and student t test at $P<0.05$.

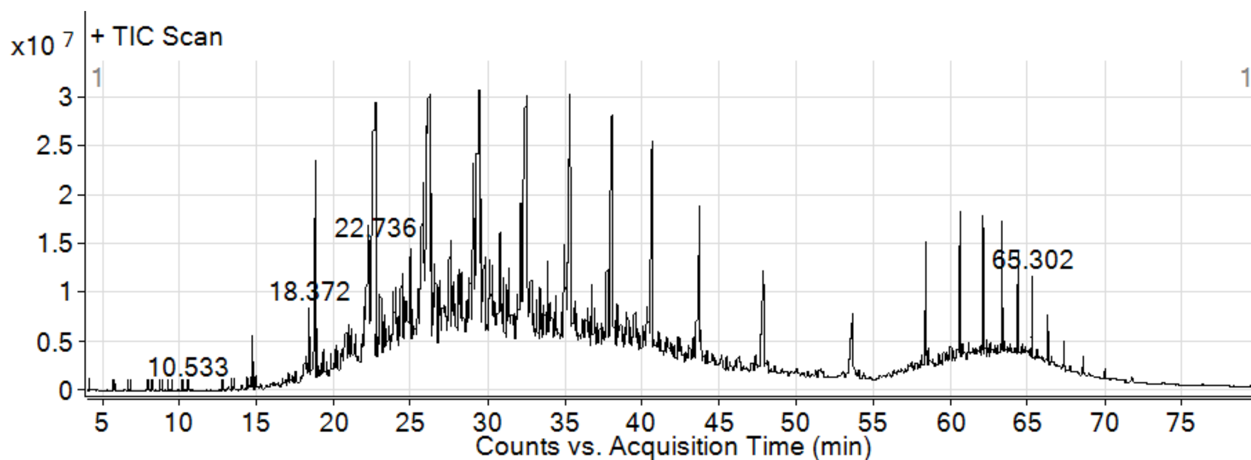


Fig. 1: GC-MS chromatogram of CME of *M. buxifolia* bark extract.

RESULTS

GC-MS and FTIR

GC-MS and FTIR chromatograms of CME (fig. 1 and 2) showed multiple characteristic peaks showed the presence of various phytochemical ingredients. The components of CME determined by GC-MS of *M. buxifolia* revealed a total of 470 compounds. FT-IR spectra represented important functional groups and are shown in fig. 2. In fig. 3 showed components identified in *M. buxifolia* by LC/MS.

Compounds and essential metals

Some of the common compounds of CME bark were presented in fig. 3. Other compounds included 2,3-Dihydro-1-methylindene, 4-Cyclopropylpyrimidine, 4-(5-Furyl)-3-hexanone, 7-Methoxyfuro[2,3-b] benzofuran, indole, serotonin,3-Hydroxymethylpyridine-2-carbonitrile, 2,5-Dimethylpyrrole-3-carbonitrile, o,o'-Bitolyl, 4,5-Dihydro-3-methyl-4-(3-methyl-1,4,2-dioxazol-5-yl)-5-phenylisoxazole, 4-Propylacridine, palmitic acid, myristic acid, and levulinic acid have been found in the CME of M. buxifolia. Essential metals identified were enlisted in (table. 1).

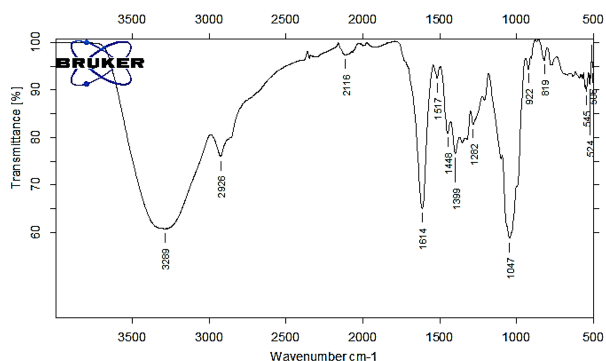


Fig. 2: FT-IR spectra of crude methanolic extract of *M. buxifolia* barks.

Isolation and purification

Compound-1: The molecular weight of compound-1 was determined by EI-MS techniques, which gives the molecular ion peak at m/z 410.1. The ^{1}H NMR (300 MHz, CDCl3) two methyl protons H-28 and H-29 were appeared as a singlet at δ 0.70 and 1.04, while the other two methyl protons H-19, 26 and H-23 appeared as doublet at 0.94 (*J*=6.5 Hz), 0.84 (*J*=6.4 Hz) and 0.84 (*J* = 6.4 Hz). The methyl proton H-24 appears as a triplet at δ 0.85 (*J*=7.2 Hz). The proton attached with alcoholic carbon showed in a downfield region at 3.53 (tdd, *J*=4.5, 4.2, 3.8 Hz). The other protons of the steroid moiety appear in the up field region i.e., δ 1.0-2.4. The spectral data confirms the compound to be stigma sterol fig. 4.

Table 1: Multiple metal detection in methanolic fraction of *M. buxifolia* barks.

Metal Name	Purity (%)	Metal Name	Purity (%)
Na	0.12	Fe	0.081
K	0.794	Mg	0.071
Zn	0.116	Ca	0.326
Mn	0.052	Si	0.176

Compound-2: The EI-MS gives the molecular ion peak at m/z 314.1 [M]+. The ^{1}H NMR (300 MHz, CDCl3) show downfield signal at δ 7.89 (d, *J*=8.8 Hz, 2 H, H-2′, 6′) and 6.93 (d, *J*=8.8 Hz, 2 H, H-3′, 5′). The two protons i.e., H-8 and H-4 appears as a singlet at δ 6.82 and 6.6. The up field methoxy protons (H-10, H-11) appear at δ 3.97 (s) and 3.82 (s) respectively. The spectral data gives the following structure and the compound elucidated is "Cirsimaritin" that is already reported fig. 5.

Antioxidant

The scavenging activity of DPPH radicals

The DPPH antioxidant potentials of the CME and its subsequent fractions were depicted in fig. 6(A). Ascorbic acid showed 89.55±1.29% DPPH free radical scavenging

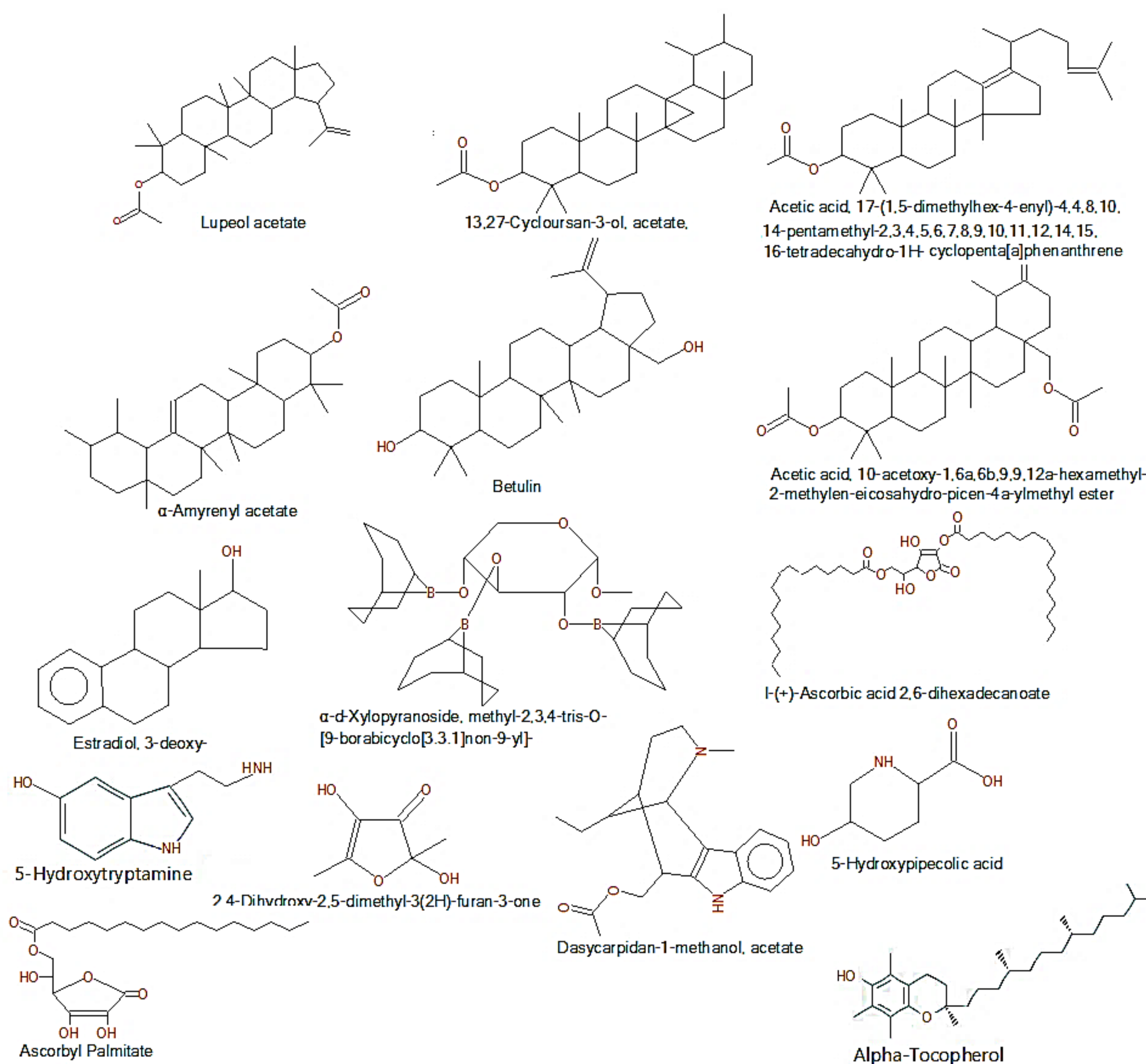


Fig. 3: Structures of various compounds identified through LC/MS analysis in CME of *M. buxifolia* barks.

at a concentration of 500µg/mL. CME and its CHF fraction scavenge 87.6±1.56 and 84.80±1.66% DPPH free radicals respectively at 500µg/mL as illustrated in fig. 6(A).

Superoxide % scavenging

Superoxide scavenging potentials of the *M. buxifolia* CME and its fractions were graphically illustrated in fig. 6(B). The CME exhibited 82.10±1.86% super oxide scavenging potentials followed by CHF 80±1.0% free radical scavenging at 500µg/mL concentration. NHF, EAF and AQF showed 66.92±1.0, 80.0±1.0 and 62.11±1.12% antioxidant activity respectively.

Hydrogen peroxide% scavenging

M. buxifolia bark extract against hydrogen peroxide showed dose dependent antioxidant activity. as shown in fig. 6(C). Ascorbic acid inhibited 81.43±2.0% H_2O_2 free radicals, while CME also showed significant antioxidant results (80.55±2.0%). In fractions antioxidant results were CHF>EAF>AQF> and NHF> respectively.

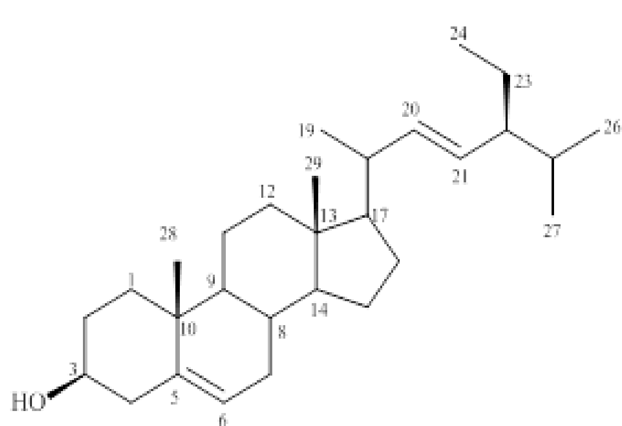
Neuropharmacological activity

Open field test

M. buxifolia CME and its fraction increase the movement activity of mice in open filed at (100, 300 and 500mg/kg, body weight) fig. 7(A). The number of squares crossed by the mice were significant at ($p<0.01$) as compared to control and diazepam as a standard at the dose of 2 mg/kg. With CME 485±3.33, NHF 338±1.36, EAF 357±1.11, CHF 363±1.78, and AQF 340±1.89 number of squares were travelled by mice at 500mg/kg dose.

Rearing

The rearing exploratory performance of mice observed for the control group was 28.33±0.6 and with CME, NHF,



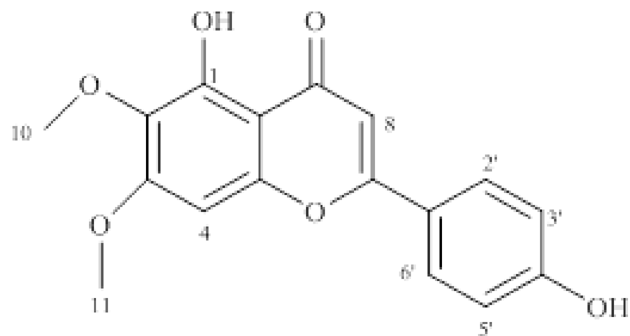


Fig. 4: Structure of compound 1: stigmasterol.

Fig. 5: Structure of compound 2: cirsimaritin.

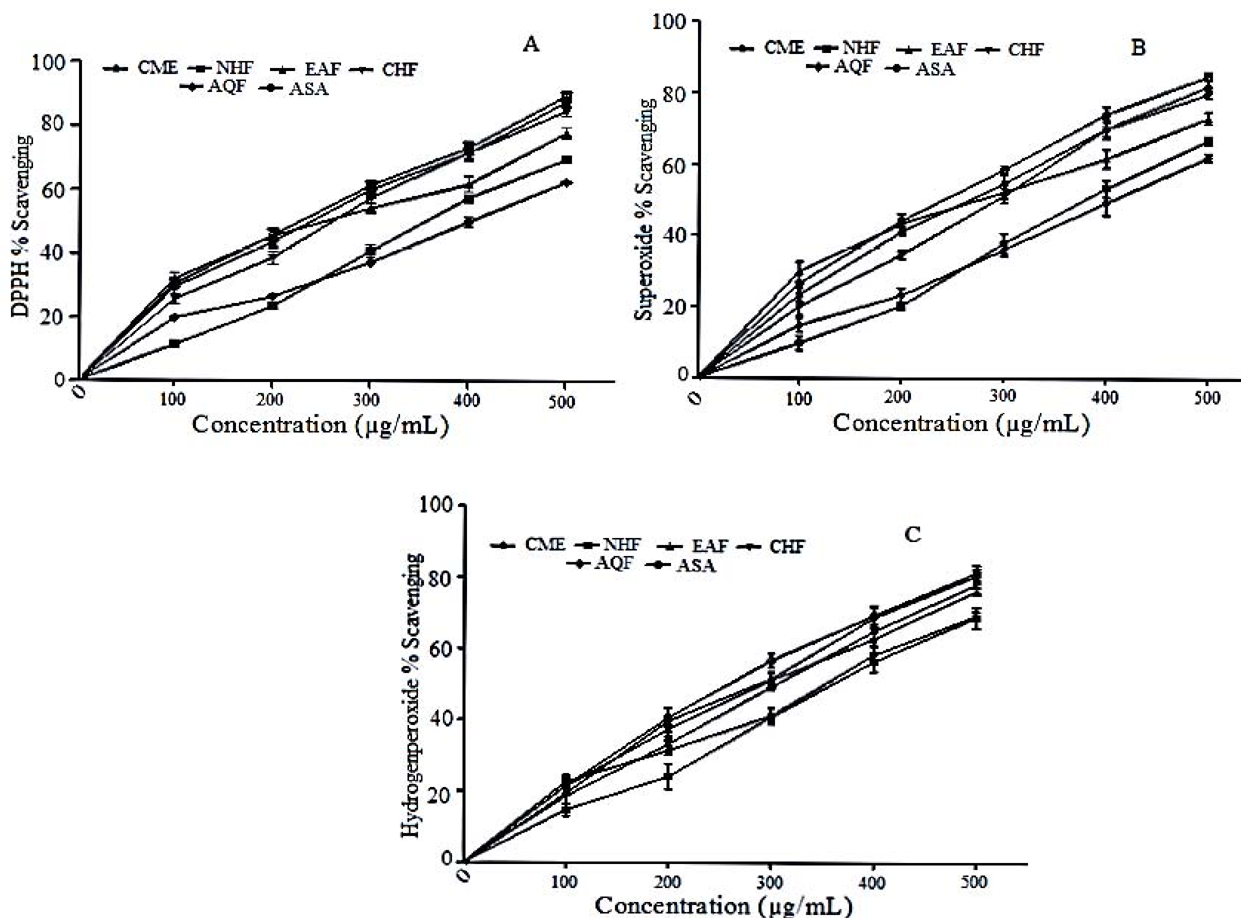


Fig. 6: Antioxidant potential *M. buxifolia* barks CME and its subsequent fractions are given at various concentrations. Each value represents a mean±S.E.M (n=3): (A)=DPPH radical scavenging, (B) super oxide scavenging (C) hydrogen peroxide scavenging activity. CME= crude methanolic extract of *M. buxifolia*; NHF=*n*-hexane fraction; EAF=ethyl acetate fraction; CHE= chloroform fraction; AQF= aqueous fraction; ASA= ascorbic acid.

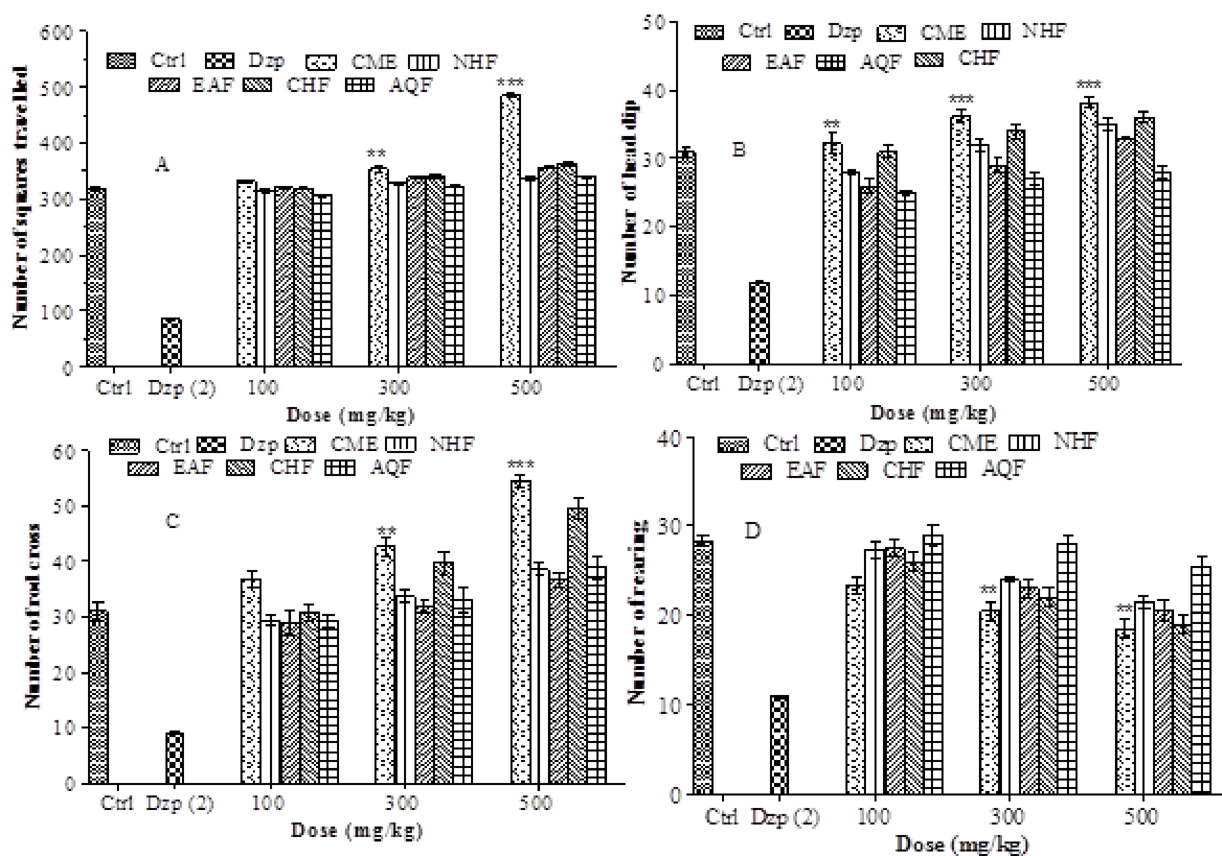


Fig. 7: Neuropharmacological outcome of CME and its subsequent fractions of *M. buxifolia* bark at different concentrations. (A) Open field test, (B) head dip test, (C) traction time, and (D) rearing activity. Each group is displayed as mean ± SD; n=6. According to ANOVA test: CME= crude methanolic extract, NHF= *n*-hexane fraction, EAF= ethyl acetate fraction, CHF= chloroform fraction, AQF= aqueous fraction of *M. buxifolia*; Ctrl was normal saline, Dzp 2mg/kg.

EAF, CHF, and AQF were (18.5±1.11, 21.5±0.75, 20.5±1.20, 19±1, and 25±1.05) respectively at 500 mg/kg dose. Diazepam (2mg/kg) treated group showed 10.83±0.22 rearing fig. 7(D).

Hole cross test

CME and its fractions at (100, 300 and 500mg/kg) dose, exhibited a significant increase in head dip test at 500mg/kg dose ($p<0.01$) fig. 7(B). On the other hand, standard (Diazepam at 2mg/kg) showed diminished head dip response. CME showed 38.16±0.83 responses of head dip, while, CHF, EAF. NHF showed 36±0.80, 33±0.08, and 35±1.0 responses of head dip at 500mg/kg dose.

Traction test

In this motor control test animals who received CME at 500 mg/kg dose cross the 1-meter rod 54.66±1.16 times/ 30 minutes ($p\leq0.05$), while at similar dose of fractions i.e. NHF, EAF, CHF, and AQF the number of rod crossed response were 38.55±1.15, 36.66±1.25, 49.6±1.75 and 38.85±2/ 30 minutes respectively. Diazepam at 2 mg/kg as showed 9±0.12 responses fig. 7(C).

Force swimming test

All doses of *M. buxifolia* CME and its fractions reduced the immobility period of mice. At 500 mg/kg with CME immobility time was 1.31±0.46 minutes (**$p<0.01$). While with NHF, EAF, CHF, and AQF 500 mg/kg the immobility time were 1.46±0.60, 1.67±0.75, 1.57±0.75, and 1.74±0.45 minutes. Imipramine 10 mg/kg significantly 0.92 minutes (**$p<0.01$) attenuate the immobility time in comparison with control group.

DISCUSSION

M. buxifollia fruits are reported for its medicinal values. However, less knowledge about the compounds present in bark, present phytochemical study has been designed. *M. buxifolia* bark extract and its fractions were screened for identification of bioactive compound via GC-MS, FTIR, and for metal analysis using ICP-OES. Furthermore, LC-MS was also carried out to see those compounds which were not screened in GC-MS. Phytochemical investigation of the plant extracts reveal that, *M. buxifolia* contains quinolone, indole derivatives, alkaloids, steroids,

tannins, anthraquinone glycosides, glycosides, and saponins. Free radicals are considered to contribute in several CNS disorders, like neuritis, epilepsy, parkinson, huntington, alzheimer disorder, aging, and atherosclerosis of one's brain (Nigam, 2015). They damage tissue by interacting with the cell membrane, DNA and proteins. Impairment of (polyunsaturated fatty acids), nucleotides in sulfhydryl bonds are key pathways of damage (Machlin & Bendich, 1987). So oxidative stress association with several disorders have developed interests in discovering bioactive compounds from medicinally potent plants. Plants are considered as an alternative source of free radical scavengers (Meena, Pandey *et al*. 2012). Currently used synthetic antioxidants like Butylated hydroxytoluene (BHT), butylated hyroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are linked with various toxicities (Imran, Ullah *et al*. 2017). Therefore, scientists focus on the natural products, to get better results (Shu, 1998). It is highly required to develop medicinal plants based economic, safe and effective treatment strategies for preventing oxidative stresses associated neurological disorders.

In this study free radicals scavenging potential of *M. buxifolia* CME bark and its fractions showed significant antioxidant against DPPH, H_2O_2 and superoxide and nearly comparable with the positive control ascorbic acid. On interaction with the free radical antioxidants prevent them to mediate damages in the body (Sies, 1997). As we observed in results that CME showed significant antioxidant activity, this may be due to the presence of antioxidants likel-(+)-Ascorbic acid 2,6-dihexadecanoate, α-tocopherol, Cirsimaritin an active flavone Fryer, (1992) as shown in the results. Stigmasterol was also presented in CHF exhibited antioxidant, anti-inflammatory, and antimutagenic property (Kaur, Chaudhary, Jain, & Kishore, 2011). Therefore, the significant antioxidant results may be due to above antioxidant compounds present in bark extracts. In an agreement to antioxidant potential of *M. buxifolia* bark extract and its fractions, it significantly affect locomotor activity. For the first time *M. buxifolia* bark extract was assessed for its neuropharmacological effect on mice. These activities were analyzed by head dip, open field, rearing, and traction tests. Results suggests that, all concentrations of *M. buxifolia* bark extract and its fractions improve the frequency of movements and generalize performance. To monitor the activity of chemical agents or drugs having effect on CNS, this model is considered as a sensitive method. CNS excitation /or sedation is reflected by locomotor activity, and behavioral changes. Locomotion frequency is to judge one's level of CNS excitation or sedation (Thirupathy, Tulshkar, & Vijaya, 2011). Numerous flavonoids and steroids are considered as GABA receptor ligands in the CNS; due to which they are suggested as benzodiazepine-like compounds (Fernández *et al*., 2006; Johnston, 2005). The increased in locomotive activities recorded in present study may be due to an interaction of the above compounds present in *M. buxifolia* bark extract with GABA, and serotonin receptors in the CNS. These effects were supported by our observation in the current study. It may be postulated that the aforementioned antidepressant action may be due to interaction its active constituent to GABA and serotonin receptors. Additionally, compounds like alkaloids and flavones also have high binding affinity for the benzodiazepine site of the GABA receptor (Hanrahan, Chebib, & Johnston, 2011). Antidepressant drugs attenuated the period time of immobility in rodents (Porsolt *et al*., 1977). Similarly, the CME of *M. buxifolia* bark extract also reduce the immobility time in mice during the FST, and increased the locomotive and exploratory activity in mice. Though the particular mechanism in the observed increased locomotive and antidepressant potential is not yet clear. However, the observed results during experiment proposed a probable direct/indirect boosting of the serotonergic signals in the CNS. The GC-MS analysis showed the presence of 5-hydroxytryptamine in the extract, that may be responsible for such activity, as 5-hydroxytryptamine is a potent neurotransmitter (Piñeyro & Blier, 1999). Further, activities on plant bark extract may provide confirmation of its mechanism of action.

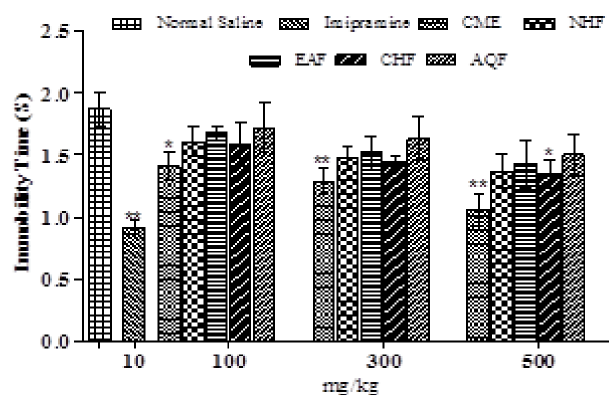


Fig. 8: Effect of imipramine and CME/fraction on immobility time period of mice subject to the FST. The data are presented as mean ± SD; n = 6. All groups were compared to neutral control group (normal saline) according to ANOVA, and Tukey post-hoc test: *$p < 0.05$, **$p<0.01$.

CONCLUSION

In the conclusion, current study demonstrated that, *M. buxifolia* possess important bioactive molecules. The appreciable antioxidant and antidepressant potential may be due to enrich source of bioactive compounds. Based on above results further investigation may be beneficial from these results.

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