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Research Article

Pharmacological and gross behavioral studies on *Memecylon terminale Dalz,* a medicinal plant from Western Ghats in southern India

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ABSTRACT

The *Memecylon terminale Dalz* is one of the important medicinal plants that are being used extensively by the Indian traditional healers to cure many diseases although there are no reports on the identity of the active ingredients. This plant belongs to the family of *Melastomataceae*, exclusively found in the Western Ghat region of Karnataka in India. In this study, we prepared the extracts of this plant by continuous Soxhlet's extraction using petroleum ether, chloroform and methanol. The concentrated extracts were assayed for their phytochemical constituents, and determined their antibacterial, analgesic, antioxidant and RBC protective activity. The phytochemical analysis of *M. terminale Dalz* extracts revealed the presence of significant levels of alkaloids and flavonoids and moderate amounts of steroids, tannins and phenols. Among the extracts, the methanolic extract of the plant, containing a good percentage of phenolics, showed a dose dependent antibacterial activity against different Gram-positive and Gram-negative bacterial strains and in addition, it was found to have a good antioxidant property and analgesic activity. *M. terminale Dalz* is an endemic medicinal plant found only in the Western Ghats of Karnataka, which has the ability to inhibit the growth of pathogenic microorganisms and possessing very good antioxidant and analgesic property. Further detailed studies are needed to identify the active principles and their relationship to biological activities.

Key Words: *M. terminale Dalz*, antibacterial activity, antioxidant activity, RBC protective activity, phenolic compounds

INTRODUCTION

Since time immemorial, various medicinal plants have been employed across the world for the treatment of variety of human ailments including infectious diseases, although there is a lack of proper documentation. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare [1]. The trend to move towards natural products from medicinal plants have gained increased attention in recent years compared to the use of synthetic drugs as they are free from side effects. The phytochemical constituents of several medicinal plants have been shown to possess novel molecules that have been proved to be effective drug molecules by rigorous science. Many phytochemicals are known to exert antimicrobial, antioxidant and analgesic property that have been recognized as beneficial to human health and for the prevention of disease [2]. The increasing prevalence of multidrug resistant strains of microbes and the reduced susceptibility to antibiotics and antifungal drugs available in the market raises the number of untreatable bacterial and fungal infections; hence there is an urgency to search for the new infection-fighting strategies [3, 4]. In this regard, efforts are being made by systematic screening of the plants to discover novel antimicrobial, antioxidant and analgesic compounds.

In India, the mountain region called Western Ghats is situated in the south extending from Kanyakumari to Maharashtra. The Western Ghat region that belongs to Karnataka state is endowed with a diverse flora and fauna of which many are strictly specific to this region. Several medicinal

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plants that are indigenous to this region have been in use by the local traditional healers to cure various diseases. These include, *Aegle marmelos* for the treatment of chronic constipation or intestinal blockage, *Bacopa monnieri* as an anti-anxiety drug and for enhancing cognitive properties which has shown positive effects on brain, *Celastrus paniculatus* tonic for treating the gout and rheumatism, *Holarrhena pubescens* bark and roots for the treatment of diarrhea and dysentery, *Centella asiatica* for treating fever and ulcer, *Atalantia monophylla* to treat chronic rheumatism and paralysis, *Dodonae aviscosa* for healing wounds and *Euphorbia hirtalinn* for curing bronchial infection and asthma [2].

The *Memecylon terminale Dalz* is an endemic plant that is exclusively present in the Western Ghats and belongs to the family *Melastomataceae*. Nearly three hundred *memecylon* species are distributed in different habitats like semi-evergreen, evergreen, deciduous and mountain shoals with a wide range of altitude from sea level. All the parts of this plant are being used to cure disorders such as dysentery, diabetes, diarrhea, piles, haemoptysis and menorrhagia and shown to possess carminative stomachic astringent property [5].

Despite its utility as an important medicinal plant, there is no data regarding the isolation and characterization of active principles present and their structure-activity relationship. In the present investigation, an attempt has been made to systematically study the extracts of *M. terminale Dalz* plant for its application in the field of pharmacy. Following preliminary phytochemical analysis, systematic pharmacological activities have been determined for different extracts of the leaves to test its usefulness as a source of antibacterial, antioxidant and analgesic agent.

MATERIALS AND METHODS

Collection and Identification of the Plant Material: The *M. terminale Dalz* plant is an erect shrub that grows at an altitude of 850 meters above the sea level and latitude of 13˚ 43' 47" to North and 75˚ 00' 38" to East in the Hulikal Ghat located in Shimoga District [6]. The plant was collected at the flowering stage and the identification and authentication of the plant material were made at the Department of Botany, Kuvempu University, Shankaraghatta, Shimoga, Karnataka, India. The voucher specimen has been deposited in the herbarium of Department of Botany, Kuvempu University. The collected plant material was thoroughly washed with distilled water followed by 30% ethanol, shade dried and mechanically grinded into fine powder and stored in a dry place until further use.

Preparation of the plant extracts: A known weight of the finely crushed powder was successively extracted with the solvents of increasing polarities (i.e., petroleum ether, chloroform and methanol) by using Soxhlet's apparatus. The apparatus was made to run for 48 cycles or until the solvent becomes colorless in the thimble. The weight of powder was recorded every time before and after Soxhlet's extraction. The solvent extracts were concentrated under reduced pressure and stored at -20°C until use. The concentrated extracts were used for analyzing phytochemical constituents and determining the antioxidant, antibacterial and analgesic activities.

Phytochemical Analysis: The phytochemical analysis of all the three extracts was carried out according to the procedure of Indian Pharmacopoeia [7]. The phytochemical constituents such as alkaloids, flavonoids, steroids, tannins, phenolics, saponins, proteins and amino acids were estimated both qualitatively and quantitatively for all the extracts as described earlier [8]. Quantitatively, total phenolic content was determined using Folin-Ciocalteu's reagent and expressed as catechol equivalents [9], total flavonoids content was analyzed by modified method of Zhishen et al. [10] and the results expressed as catechin equivalents (mg catechin/g dried extract).

Bacterial strains and Antibacterial assay: *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were used for determining the antibacterial activity. All bacterial strains were maintained on nutrient agar medium at 37°C. The *in vitro* antibacterial activities of the extracts were tested against Gram +ve and -ve bacteria by well plate technique at different concentrations (1, 0.5, 0.25 and 0.12 mg/ml). 0.1ml of 24 h old bacterial suspension (10–100 cfu/ml) was spread on nutrient agar plate and the wells made by using a sterile cork borer. The extracts in DMSO (60µl) were added to respective wells and incubated for 24 h at 37^{o}C. After the incubation, the zone of inhibition was measured (in mm). The minimum inhibitory concentration (MIC) of the extracts was determined with the broth dilution method using nutrient broth. The MIC value, representing the lowest concentration that completely inhibited the formation of visible growth, was evaluated after 18 h of incubation at 37°C.

Antioxidant activity: There are several methods for the determination of antioxidant activity of plant extracts as they often contain mixture of compounds with different functional groups, polarity and chemical behavior, that could lead to scattered results depending on the test employed. Due to this reason, we carried out multiple assays for evaluating the antioxidant property of the extracts.

The free radical scavenging assay was performed according to the previously reported procedure by using stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) [11]. The metal chelating activity of different extracts was carried out using the procedure of Dinis et al. [12] using EDTA as standard. The reductive ability of the plant extracts was determined according to the method of Oyaizu [13]. The ability of the extracts to scavenge H_2O_2 was determined according to the method of Ruch et al. [14]. The total antioxidant activity of the test sample was assayed by the phosphomolybdenum method as described by Prieto et al. [15]. The total antioxidant capacity was expressed as ascorbic acid equivalents (AAE).

Animals: Adult male Swiss Albino mice, 6 to 8 weeks old, weighing 22-25 g, obtained from the Veterinary College, Bangalore, India, were used in this study. The animals had free access to standard pellet chow food and water and they were housed under natural (12h each) light-dark cycle. The animals were acclimatized for 7 days to the laboratory conditions before performing the experiments. The experimental protocol was approved by the institutional animal ethical committee (Reg. No-628/02/c/CPCSEA).

Acute Toxicity and Gross Behavioral Studies: Acute oral toxicity assessment for the extracts was carried out according to Organization for Economic Co-operation and Development (OECD) guidelines No. 420 [16]. Each group consisting of 6 mice (overnight fasted) was housed in the colony cage at 25±2°C with 55% relative humidity and 12 h of light and dark cycle. A single dose extract of 125, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight was administered orally as a fine suspension in saline and gum acacia powder. The symptoms of acute toxicity and behavioral changes because of test compounds were observed and recorded continuously at an interval of 4 h for the next 24 h [17,18].

Analgesic Activity: The central analgesic activity of the extracts was studied against thermal stimuli using the hot plate according to the procedure of Eddy and Leimbach [19]. The albino mice were divided randomly into 13 groups, each group consisting of 6 mice. Group 1 was control (vehicle only, 10% Tween 80 in distilled water), groups 2-4 treated with the standard and groups 5-13 administered orally with different concentrations (100, 150 and 200 mg/kg body weight) of petroleum ether, chloroform and methanol extracts, respectively. The initial reaction time of control and test group animals was recorded by placing them on the hot plate (55±0.5°C) and the licking of paw or jumping was taken as the index of reaction of heat. The post-treatment reaction time of each animal after the administration of plant extracts was recorded at 30, 60 and 90 min.

The peripheral analgesic activity of the extracts was evaluated using the acetic acid-induced writhing test [20]. Mice were divided into five groups containing six in each group. Group 1 served as control and administered only with the vehicle (10% Tween-80 in distilled water). Group 2 was administered with standard aspirin (100 mg/kg) and groups 3-5 were administered with single oral dose of petroleum ether, chloroform and methanolic extracts (100 mg/kg), respectively. These extracts were administered orally one hour prior to intra-peritoneal injection of 0.6% v/v acetic acid and after five minutes of post injection, the number of writhing was counted for the next 20 min.

***In vitro* assay for inhibition of human erythrocytes hemolysis:** The inhibition of human erythrocytes hemolysis was evaluated according to the procedure described by Tedesco et al. [21] with some modifications. For this, the washed RBC suspension was prepared according to the procedure of Manna et al. [22]. After obtaining informed consent, the blood samples were collected in heparinized tubes by vein puncture from healthy volunteers and the samples were centrifuged at 2,000 rpm for 5 min at 4° C to remove plasma and buffy coat. The RBCs were washed thrice with phosphate buffer saline (PBS, 125 mM NaCl and 10 mM sodium phosphate, pH 7.4) and finally re-suspended in PBS (2% v/v).

To study the hemolysis induced by the plant extracts and standard butylated hydroxyanisole (BHA), erythrocytes were pre-incubated with 50 µl of plant extract corresponding to 50 µg of AAE for 1 h and the hemolysis was determined. The percentage of hemolysis was calculated by taking hemolysis caused by 100 µl of 100 µM H_2O_2 as 100%.

***In vitro* assay for the inhibition of lipid peroxidation on erythrocytes ghost membrane:**

For inhibition assay of lipid peroxidation, the erythrocytes ghost membrane was prepared by following the procedure of Fairbanks et al. [23] and the protein content in the membrane was determined according to the method described by Lowry et al. [24] using BSA as a standard. The erythrocyte ghost membrane lipid peroxidation was performed according to the method of Stocks and Dormandy [25] with slight modifications. The plant extracts containing 10-50 µg AAE was added to 1 ml of erythrocyte ghost membrane suspension (200 mg protein) and lipid peroxidation was induced by the addition of 100 µl of 200 µM H_2O_2. The appropriate blanks and controls were run along with the test samples. The percentage of inhibition was calculated and plotted against the concentration of the samples.

RESULTS AND DISCUSSION

Preliminary Phytochemical analysis: The preliminary phytochemical investigation indicated the presence of different classes of compounds such as alkaloids, flavonoids, steroids, tannins and phenolics in the extracts of *M.* terminale *Dalz*. The methanolic extract had high alkaloids with significant amount of flavonoids, phenolics and steroids. On the other hand, chloroform extract contains significant amount of alkaloids, flavonoids and less amount of phenolics, whereas petroleum ether extract contained small amount of steroids (Table 1). Protein and amino acids were found to be absent in all the extracts. Similar results have been reported for different *Mememcylon* species such as *M. malabaricum*, *M. umbellatum burm* and *M. edule* [26-28].

Total Phenolic and Flavonoids content: The phenolic compounds constitute a major group of compounds, which act as primary antioxidants or free radical terminators in plants [29]. Their antioxidant effectiveness depends upon the stability in different systems, as well as the number and location of hydroxyl groups [30]. The total phenolic content of *M. terminale Dalz* was found to be 32.6±0.7 mg GAE/g, 45.3±0.5 mg GAE/g and 61.9±0 mg GAE/g in petroleum ether, chloroform and methanolic extracts, respectively (Table 2).

The flavonoids are the plant's secondary metabolites that include flavanols, flavones and condensed tannins etc. Similar to phenolics, the total flavonoids content was also found to vary with the solvent used for extraction. The flavonoid content was found to be 37.9±0.3 mg GAE/g in the methanolic extract, which is high as compared to chloroform and petroleum ether extracts with 20.3±0.2 mg and 13.4±0.3 mg GAE/g, respectively.

Antibacterial activity: The antimicrobial activity of different extracts of plant was tested against different strains and the results showed that *S. aureus* (50 µg MIC) was the most susceptible, and *E. coli* (100 µg MIC) was the most insensitive strain among all the bacteria used in this study. The methanolic extract of the plant was found to be active against all bacterial strains (Table 3). The petroleum ether extract showed weak activity (425 µg/ml MIC) as compared to chloroform (200 µg/ml MIC) and methanolic extracts (100 µg/ml of MIC) against *E. coli* (Table 4). In recent days, Gram-negative bacteria are frequently being reported to have developed multi drug resistance to many of the antibiotics that are currently available in the market of which *E. coli* is the most prominent [3,4]. This could probably be the reason for the high MIC values for *E. coli* as compared to *S. aureus*.

In general, among the tested microbial strains, Gram-positive bacteria are highly inhibited as compared to Gram-negative bacteria. The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be due to the difference in morphological constitutions between these microorganisms; Gram-negative bacteria having an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the bacterial cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria, on the other hand, are more susceptible due to the presence of outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram negative organisms which are more complex than the Gram positive ones act as a diffusional barrier and making them less susceptible to the plant extracts than Gram-positive bacteria [31, 32].

DPPH scavenging activity: The stable DPPH radical scavenging model is a preferable method to evaluate the antioxidant ability of plant extracts in a relatively short time compared with other methods. In this assay, standard BHT showed a strong scavenging activity (IC_{50}=18 µg), while the methanolic extract scavenges DPPH free radicals in a concentration dependent manner (~13 % at 10 µg, 61% at 50 µg and 91% at 100 µg) with an IC_{50} value of 43 µg. Among different extracts, only the methanolic extract showed good antioxidant property due to the presence of high phenolic and flavonoid contents (Fig 1).

Metal chelating activity: The metal chelating activity of *M. terminale Dalz* extracts was evaluated against Fe^{2+} ion to estimate the potential antioxidant activities of the different extracts. The

results indicate that methanolic extract exhibits good metal chelating activity when compared to other two extracts (Fig 2). The chelating activity of methanolic extract at 100µg/ml was found to be 76.6±0.3% as compared to 29.8±0.2% and 48.3±0.4% for petroleum ether and chloroform extracts, respectively.

Total reductive capability: The activity of potent antioxidants has been attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. In this study, as shown in Fig. 3, the extracts exhibited higher antioxidant activity as shown by the absorbance values at 700 nm. The methanolic extract showed stronger reducing power (0.73±0.04) as compared to chloroform extract (0.49±0.03) and petroleum ether extract (0.16±0.08) whereas the standard ascorbic acid showed an absorbance value of 1.23±0.04 at 100µg/ml (Fig 3). As in the case of DPPH activity, the reducing power of these extracts also showed concentration dependence.

Hydrogen peroxide scavenging activity: In this study, ability of different extracts of *M. terminale Dalz* to scavenge hydrogen peroxide was evaluated at a concentration of 100µg/ml. The percentage hydrogen peroxide scavenging effect exerted by the methanolic extract was found to be 67.4±0.2 as compared to 86.1±0.9 for ascorbic acid, both at 100µg/ml concentration. On the other hand, petroleum ether and chloroform extracts also showed moderate peroxide scavenging activity (Fig 4).

Total antioxidant activity: The total antioxidant capacity of different extracts of *M. terminale Dalz* was evaluated by the phosphomolybdenum method and expressed as number of ascorbic acid equivalents (Table 5). From the data, it is evident that methanolic extract has high total antioxidant activity value of 47.6±0.4 AAE when compared to other two extracts (petroleum ether extract 22.8 ±0.8 AAE and chloroform extract 27.2 ±0.3 AAE).

Analgesic activity: The effect of different extracts of *M. terminale Dalz* on acetic acid-induced writhing in mice was evaluated and shown in Table 6. The results indicate that the methanolic extract is highly effective as an analgesic at 60 and 90 min at a concentration of 200 mg/kg body weight when compared to other two extracts. The methanolic extract significantly reduces the writhing induced by the acetic acid after oral administration in a dose dependent manner and is comparable with that of reference drug Pentazocine. After oral administration (200 mg/kg bodyweight), the percent inhibition was 82.5±0.9% for standard Pentazocine, 30.2±0.3% for petroleum ether extract, and 46.1±0.2% for chloroform extract and 71.6±0.2% for methanolic extract. The peripheral analgesic activity of petroleum ether, chloroform and methanolic extracts of *M. terminale Dalz* against acute inflammatory pain was also determined and shown in Fig 5. The results indicate that methanolic extract has very good pain reliving property as that of standard drug Aspirin, when compared to other extracts. The drugs such as Aspirin and Pentazocine inhibit the formation of pain signal producing substances in the peripheral tissues resulting in the relief of body pain. Therefore, it is likely that *M. terminale Dalz* extracts might suppress the formation of these substances or antagonize the action of these substances and thus exerting analgesic activity in acetic acid-induced writhing test and in hot plate test. On the other hand, the plant extracts significantly increased the reaction time in hot-plate test, suggesting its central analgesic activity. The analgesic response is mediated by peritoneal mast cells, prostaglandin pathways and sensing of ion channels. Flavonoids increase the endogenous serotonin or interact with the 5-HT2A and 5-HT3 receptor, which is involved in the mechanism of central analgesic activity [33]. Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it is suggested that reduced availability of prostaglandins in the presence of flavonoids of plant extracts might be responsible for its analgesic effect.

Acute Toxicity: The toxicity studies in mice revealed that all the extracts of the plant are quite safe in the concentration range of 500-3000 mg/kg body weight and no significant gross behavioral changes or death of experimental animals were observed. Further, above the concentration of 4000 mg/kg body weight, the experimental animals showed depression during the first four hours and death after 20th hour.

Inhibition of human erythrocytes hemolysis: Many natural and synthetic compounds have the ability to prevent the hemolysis of erythrocyte induced by H_2O_2 [21]. Initially, we studied the effect of different extracts of *M. terminale Dalz* plant on human RBC membrane and the results confirm that plant extracts alone do not have any detrimental effect on erythrocyte membranes. The percentage of hemolysis in the presence of extracts was found to be in the range of 2.3 to 3.1% which is comparable to the control (1.9%). The methanolic extract of *M. terminale Dalz* plant

showed good inhibition activity against human erythrocytes hemolysis when compared to other two extracts (Fig. 6). The inhibition efficiency of hemolysis of human erythrocyte by the plant extract was found to be concentration dependent. The mechanism of membrane protective activity can be explained by membrane bilayer couple hypothesis model [34]. This hypothesis model has been proposed to explain the interactions of any foreign molecule with membrane and different expansion of the lipid portions of the two surfaces of the RBC membrane. It is possible that other perturbations, by acting on the protein components of the membrane, may also lead to a differential expansion of the two surfaces [35]. Thus, stomatocytes are formed when the compound inserted into the monolayer, whereas speculated-shaped echinocytes are produced when it locates into outer moiety [34]. The compounds that are produced during this process are neutralized by the plant extracts resulting in the normal structural architecture of the RBC membrane.

Inhibition of lipid peroxidation on erythrocytes ghost membrane: The lipid peroxidation produces extremely reactive product mixture such as lipid peroxides and this is a common process in all the biological systems, which has a deleterious effect on the cell membrane, proteins and DNA [36]. Malondialdehyde is one of the major products of lipid peroxidation, which is used as a marker for oxidative stress. In Fig. 7, we have shown that the lipid peroxidation could be effectively inhibited by the extracts of *M. terminale Dalz*. Further, only methanolic extract showed higher inhibition on lipid peroxidation, which may be attributed to its high phenolic content.

CONCLUSIONS

M. terminale Dalz is an endemic medicinal plant found in the Hulikal region of Western Ghats in Karnataka, which is being used by the traditional healers to treat a variety of diseases. However, in the literature, there is no reported systematic study to correlate the structure-function relationship. In this study, the plant extracts have been analyzed for its phytochemical content and were tested for their various biological activities. Among the three different extracts, methanolic extract showed highly promising biological activities including analgesic and antioxidant activities, which can be attributed to the presence of significant amount of alkaloids, flavonins, steroids and phenolics as evident from its phytochemical contents. These results indicate that *M. terminale Dalz* has a rich source of novel biologically active compounds and a detailed systematic study aiming towards the isolation and characterization of these novel molecules of biological importance is needed to further substantiate our findings.

ACKNOWLEDGEMENTS

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Table 1: Phytochemical analysis of *M. terminale Dalz* extracts

Tests	Petroleum ether	Chloroform	Methanol
Alkaloids:			
i. Mayer's test	-	++	+++
ii. Dragendorff's test	-	++	+++
Flavonoids:			
i. NaOH solution test	+	++	++
ii. Lead acetate test	+	++	++
Steroids:			
i. Salkowski's test	++	-	++
ii. Libermann-Burchard's test	++	-	+
Tannins &phenolics			
i. Lead acetate test	-	+	++
ii. Dilute HNO_3 test	-	+	++
Saponins:			
i. Foam test	-	-	-
Proteins & amino acids			
i. Biuret test	-	-	-
ii. Xanthoproteic test	-	-	-

The phytochemical constituents present in different extracts of *M. terminale Dalz* (- Absent; + Present; ++ Significant; +++ Highly significant)

Table 2: The percentage (g/100g) of total phenolics and flavonoids content of *M. terminale Dalz* extracts (as Catechol equivalent)

Extract	Total Phenolics (% w/w)	Total Flavonoid (% w/w)
Petroleum ether	32.6±0.7	13.4 ± 0.3
Chloroform	45.3±0.5	20 .3± 0.2
Methanol	61.9±0.5	37 .9± 0.3

Table 3: Antibacterial activity of *M. terminale Dalz* extracts

Extracts	**Concentration (mg/ml)**	***Escherichia coli***	***Staphylococcus Aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
		Zones of inhibition(mm)*			
Standard Streptomycin	01	16.2±0.2	15.2±0.2	20.8±0.3	13.2±0.2
	0.5	13.8±0.4	12.1±0.2	11.2±0.2	9.8±0.4
	0.25	11.9±0.4	9.0±0.2	10.3±0.4	6.9±0.4
	0.12	10.1±0.2	7.0±0.2	07.1±0.5	5.8±0.4
Petroleum ether	01	3.2±0.1	2.0±0.2	1.0±0.1	4.3±0.2
	0.5	2.3±0.2	1.2±0.1	--	3.1±0.3
	0.25	--	--	--	1.1±0.1
	0.12	--	--	--	--
Chloroform	01	3.2±0.2	5.9±0.3	8.1±0.1	8.2±0.3
	0.5	2.2±0.2	4.8±0.3	5.5±0.4	6.0±0.2
	0.25	1.2±0.3	4.0±0.2	3.3±0.2	5.2±0.2
	0.12	--	2.3±0.2	1.2±0.2	3.2±0.4
Methanol	01	9.2±0.2	12.3±0.2	10.2±0.2	10.4±0.4
	0.5	7.3±0.4	8.4±0.3	8.1±0.2	7.2±0.2
	0.25	5.6±0.5	6.3±0.2	7.1±0.2	6.2±0.3
	0.12	3.6±0.5	5.0±0.2	5.8±0.5	4.7±0.5

*****The antibacterial activity of different extract of *M. terminale Dalz* as expressed by the zone of inhibition (in mm). The experiment was performed in triplicate and the values expressed are as Mean ±SD.

Table 4: Minimum inhibition concentration of *M. terminale Dalz* extracts

Extracts in µg/ml	***Escherichia coli***	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
Petroleum ether	425	550	750	325
Chloroform	200	125	125	175
Methanol	100	50	50	100

The experiment was performed in triplicate and the values expressed are as Mean ±SD.

Table 5: Total Antioxidant activity of *M. terminale Dalz* Extracts

Sl no	Extract	Total Antioxidant Activity equivalent to ascorbic acid
1	Petroleum ether	22.8 ±0.8
2	Chloroform	27.2 ±0.3
3	Methanol	47.6±0.4

The experiment was performed in triplicate and the values expressed are as Mean ±SD.

Table 6: Effect of *M. terminale Dalz* plant extracts on acetic acid-induced writhing test mice.

Treatment	% inhibition		
	100	150	200
Aspirin	51.1±0.6	65.4±0.8	82.5±0.9
Petroleum ether extract	16.0±0.4	23.8±0.7	30.2±0.3
Chloroform extract	30.2±0.3	38.6±0.5	46.1±0.2
Methanol extract	47.4±0.9	59.3±0.4	71.6±0.2

The experiment was performed in triplicate and the values expressed are as Mean ± SD. n=6.

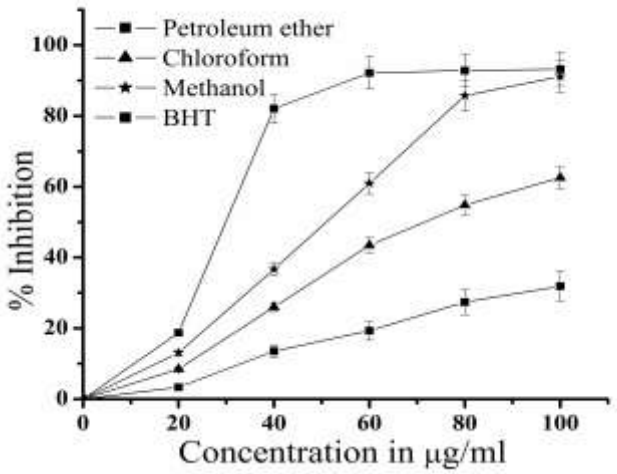

Fig 1: The DPPH scavenging activity of different extracts of the plant, *M. terminale Dalz,* in comparison with the standard.

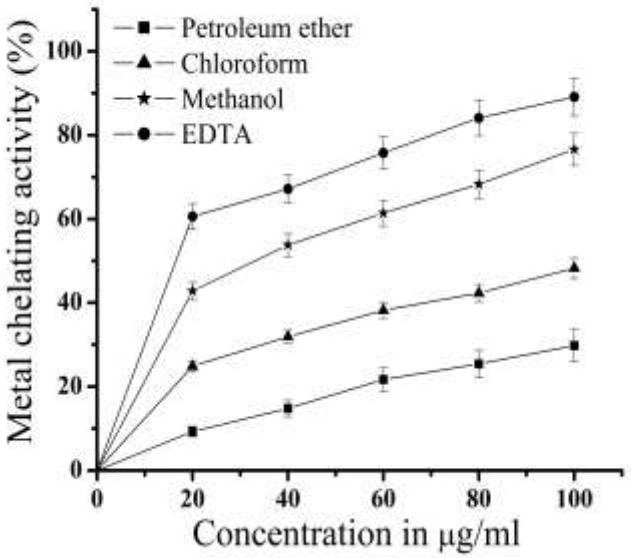

Fig 2: The metal chelating capacity of different extracts of the plant, *M. terminale Dalz,* in comparison with the standard.

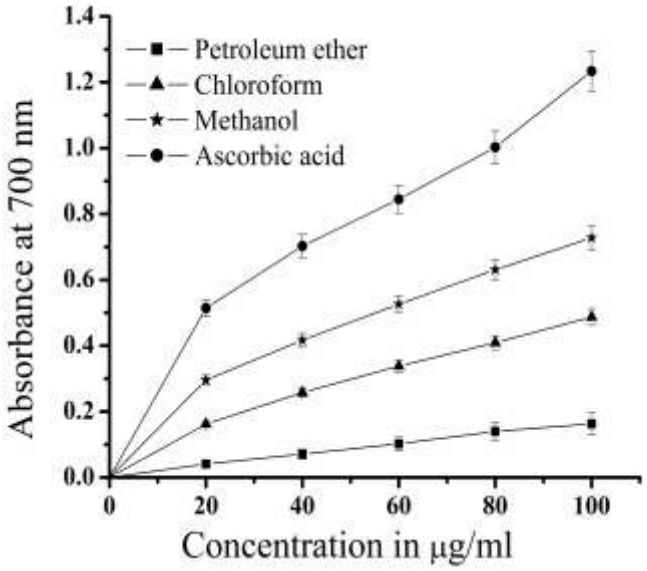

Fig 3: The reducing power assay of different extracts of the plant, *M. terminale Dalz,* in comparison with the standard.

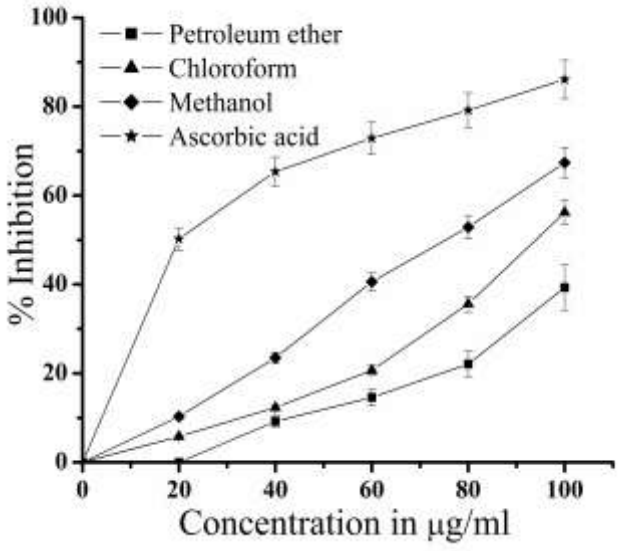

Fig 4: The percentage hydroxyl radical scavenging activity of different extracts of the plant, *M. terminale Dalz,* in comparison with the standard.

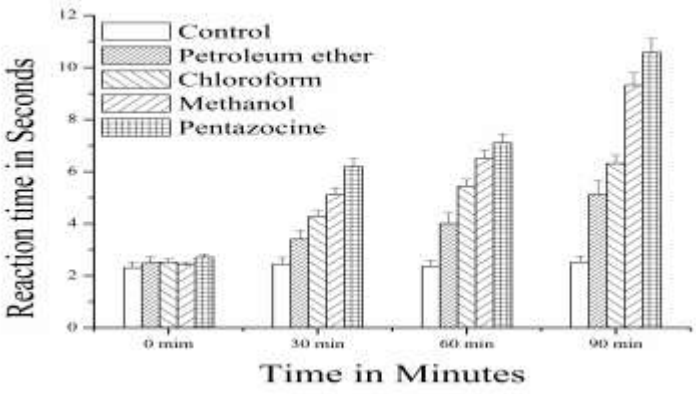

Fig 5: Analgesic effect of petroleum ether, chloroform and methanol extract of *M. terminale Dalz* on heat stimulation response in the hot plate test. Values are the mean ± SEM (n = 6), control (vehicle) – 10 % Tween 80 in distilled water; standard drug – pentazocine.

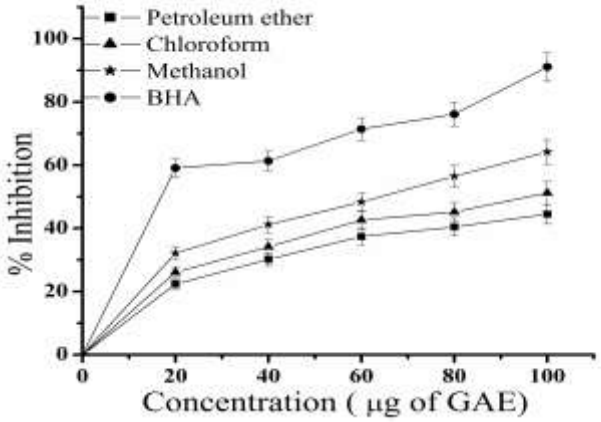

Fig 6: In vitro protective effects of *M. terminale Dalz* extracts against H_2O_2 induced hemolysis of human erythrocytes.

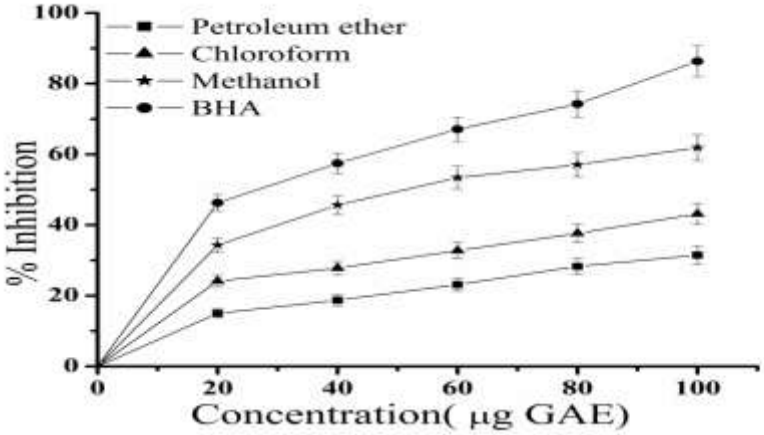

Fig. 7: *In vitro* protective effects of *M. terminale Dalz* extracts against H_2O_2 induced lipid peroxidation on erythrocytes ghost membrane.

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