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A study of in-vivo antidepressant, antidiarrheal and ex-vivo thrombolytic activities of methanol extract of Mikania micrantha leaves

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Abstract
Mikania micrantha (Asteraceae) has useful properties in the field of medicine. This study is aimed to elucidate the therapeutic potential of crude methanol extract of Mikania micrantha (MEMM) leave, on Swiss mice. The tests for antidepression potential were carried out using the model of both in-vivo forced swimming test (FST) and tail suspension test (TST). Again, the anti-diarrheal efficiency was carried out through the castor oil-induced diarrheal model; the clot-lytic property was investigated by thrombus dissolution property. The test samples showed dose-dependent (200 mg/kg BW, 400 mg/kg BW) potentiality in antidepressant, anti-diarrheal and thrombolytic activities. MEMM demonstrated a significant increase in swimming tone and mobility in FST and TST. MEMM 400 (mg/kg; b.w.; p.o.) exhibited a significant reduction of diarrhoea over 4 hours. Besides, MEMM produced promising (400 (mg/kg; b.w.; p.o) 43.76 %) clot lysis activity. The observed results of this scientific investigation revealed the possibility of the suitable use of this plant and whether this plant is useful as a source of alternative medicine and new therapeutics.

Keywords Anti-depressant; Anti-diarrheal; Castor oil; Loperamide; Mikania micrantha.

Paper type Research paper

1. Introduction
Plant-based drugs are considered cheaper and less antigenic than commercially synthesized drugs. In Bangladesh, people from urban areas utilize medicinal plants for treating a variety of disease conditions (Mollik, Hossan, Paul, Taufiq-Ur-Rahman, Jahan, & Rahmatullah, 2010). Plants and microbes remain a good source of active compounds which can be utilized as medicines due to their diversity of having potential bioactive molecules (Cragg & Newman, 2013). As these plants consist of potentially therapeutic agents, these plants have taken a significant share within several raw materials used in modern medicines (Dias, Urban, & Roessner, 2012). Almost all parts of plants like leaves, roots, flowers, seeds and others contain bioactive

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secondary metabolites that have shown promising activities in curing variety of human health conditions (Salehi et al., 2018; Yuan, Ma, Ye, & Piao, 2016). As a result, the search for novel, safe and effective bioactive compounds from plant origin encouraged natural product chemists to turn their attention to the field of traditional medicine research.

Anxiety and other related depression constitute a major common class of heterogeneous psychiatric illnesses with no definitive curative intervention available up to this point (Fajemiroye, da Silva, de Oliveira, & Costa, 2016). Depression can be defined as alterations of mood that might range from mild symptoms to severe mood disorder that can be expressed as delusions and/or hallucinations. The Diagnostic and Statistical Manual of Mental Disorders describes the emotional states of depression with some mental states as the physical symptoms of depression such as guiltiness, sadness, suicidal tendency, lack of interest, sleep disorders, gastrointestinal disorders, changes in appetite, and changes in psychomotor function. According to the World Health Organization International Consortium of Psychiatric Epidemiology (WHO-ICPE) it is estimated that 6.3 to 15.7% of the world's population suffers from depression once in life. In addition, a lifetime prevalence of depression is found almost two-fold greater in case of female compared to male. In other statistics by the World Health Organization (WHO), 322 million people have clinical depression, among which 27% is from south east Asian region (World Health Organization, 2017). World Health Organization enlisted depressive disorder as a non-fatal disease with substantial consequences (World Health Organization, 2017). Depression can be the sole cause of severe physical disability which can disrupt the daily activities of a person along with greater morbidity and mortality rate. The other worldwide widespread psychiatric state is anxiety (Möller, Bandelow, Volz, Barnikol, Seifritz, & Kasper, 2016). Though the signs and symptoms of anxiety constitute emotional states similar to normal emotional behaviours, it may be considered a disease of psychological state if happens repeatedly with increased frequency. A variety of signs and symptoms develop when anxiety is associated with depression; notable symptoms include an increased risk of suicide, decreased response to treatment intervention and a decline in real prognosis (Kara, Yazici, Güleç, & Unsal, 2000). The most accepted hypothesis regarding the development of depression is oxidative stress and its related consequences (Michel et al., 2007). As mentioned previously, though being the second leading cause of disability, mood disorders are difficult to treat and drugs used to treat those conditions have around 60% success rate. Treatment for depression with significant improvement in signs and symptoms requires long-term

treatment with antidepressants which results in further complications on the way of getting positive outcomes (Wong & Licinio, 2001). In addition, a large portion of affected individuals does not respond well to currently approved treatments for depression which necessitates the development of new therapeutics for depression. A large number of antidepressant compounds are now approved which act via different mechanisms affecting dopaminergic and/or noradrenergic and serotonergic systems (Elhwuegi, 2004). Naturally occurring bioactive compounds in medicinal plants may play an effective role in treating depression and in the past decade those compounds have had notable applications in such case (Zhang, 2004).

Diarrhoea might be defined as a condition with increased and frequent bowel movements resulting in wet stool along with abdominal cramps (Ezekwesili, Obiora, & Ugwu, 2004). It is world's one of most lethal diseases causing substantial morbidity and mortality in paediatric patients- and this disease has more severe implications in case of malnourished children in developing countries (Chitme, Chandra, & Kaushik, 2004). Although there is a notable reduction in the worldwide morbidity and mortality in the case of diarrhoea, this disease still causes more than 2 million deaths per year- and other than deaths diarrhoea also results in reduced/impaired physical and cognitive development in countries where healthcare facilities are limited (Mehmood, Siddiqi, & Gilani, 2011; Thielman & Guerrant, 2004). Around 17.5 to 21% of children under the age of 5 die from diarrhoeal disease and this accounts for around 1.5 million deaths calculated annually (Boschi-Pinto, Velebit, & Shibuya, 2008). Regions of South-East Asia and Africa have majority of child deaths among all child deaths resulting from diarrhoea (Boschi-Pinto, Velebit, & Shibuya, 2008). Although repeated measures have been taken to eradicate this health issue, still the incidence and prevalence of diarrhoea is approximately 7.1 million per year which is quite high. In 2019, WHO listed diarrhoea as the second leading cause of reduction of life expectancy by 1.97 years which takes its position just below the statistics for lower respiratory tract infections (2.09 years). Additionally, in the year 2016, 0.9 million people died including 470,000 infant deaths by diarrhoea resulting from poor sanitation, drinking unhealthy water and unsanitary environment; this crisis was dealt by governments and even world organizations. Contaminated foods or drinks can directly spread the infection or the infection can even be transmitted from person to person (World Health Organization, 2016). Current treatment for diarrhoea includes antibiotics but those antibiotics sometimes aggravate adverse effects. On the other hand, without antibiotic the therapy sometimes results in common adverse effects like constipation (Kouitcheu, Penlap, Kouam, Ngadjui, Fomum, & Etoa,

2006). Therefore, there has arisen a need to look for new and effective drugs obtained from plant sources which always remain an essential source of novel drugs. The study and finding of the prevention and treatment of diarrheal diseases are also being encouraged by the World Health Organization (WHO) utilizing traditional medicines (Snyder & Merson, 1982).

Thrombosis is a condition of formation of the blood clot within blood-carrying vessels that might arise due to a number of pathological conditions. The resulting clot may reduce or even completely obstruct blood flow through the blood vessels and this acute condition may result in sudden morbidity and mortality (Uddin et al., 2020). Acute myocardial infarction and haemorrhage inside the brain can result from the effect of thrombosis (Emon, Jahan, & Sayeed, 2020). Disorders of thromboembolic origin account for one of the prime causes of morbidity and mortality in Bangladesh which is a common scenario in developing countries (Islam & Majumder, 2013). Due to the safety, potency and viability intravenous administration of heparin remains the first-line medication for the therapy of thrombus (Prasad, Kashyap, Deopujari, Purohit, Taori, & Daginawala, 2006). Other effective and alternative thrombolytic agents which are in current use include urokinase, streptokinase, tissue plasminogen activator (tPA), recombinant tPA and alteplase. Although streptokinase and urokinase are frequently prescribed for the treatment of thrombosis, these drugs have a number of complications including profuse bleeding, re-infarction and re-occlusion. Some serious adverse effects such as anaphylactic reactions, haemorrhage and systemic fibrinolysis are also associated with these therapies. At present, all of the available agents to treat thrombosis have one or more severe shortcomings such as bleeding tendency, an unusually large dose to achieve effectiveness and low fibrin specificity (Haines & Bussey, 1995; Rahman et al., 2013). Herbal drugs or herbal preparations are utilized or applied for the treatment of different ailments since ancient times (Demrow, Slane, & Folts, 1995). Because of the importance of the development of safer and more effective thrombolytic agents, the search for such agents from natural sources either from plants or animals seems to be a viable alternative. Substantial efforts have been made to discover, isolate and develop new natural bioactive compounds from plants or animals having anticoagulant, thrombolytic, antiplatelet and antithrombotic properties (Rajapakse, Jung, Mendis, Moon, & Kim, 2005). There is evidence from epidemiologic studies that foods having established anti-thrombotic effects can reduce the risk of thrombosis. Some progress has already been made and some herbs with thrombolytic potential have been documented (Basta, Lupi,

Lazzerini, Chiarelli, L'Abbate, & Rovai, 2004). A number of plants have been reported to possess potential thrombolytic compounds (Jakaria, Islam, Islam, Talukder, Clinton, & Ibrahim, 2017). The use of modern sophisticated laboratory facilities has made it easier to screen and isolate bioactive phytochemicals. The need for developing new potential thrombolytic agents along with the ease of quickly screening large numbers of plant-based compounds for their potential bioactivity renewed and emphasized the interest in the development of herbal drugs.

The use of conventional drugs is associated with several unavoidable adverse effects and this fact has turned the interest of recent research toward developing and discovering novel herbal compounds with pharmacological activities (Hossain et al., 2014). Many people around the world prefer medicinal plants to treat chronic illnesses, mild to moderate fever or even life-threatening diseases (Adnan et al., 2019). Traditional folkloric use of medicinal plants mainly depends on the valid and proper scientific evidence. The Asteraceae family consists of the large number of species that are rich in a variety of secondary metabolites. These compounds might be a potential source of biologically active compounds with less adverse effects and with desired pharmacological effects.

Mikania micrantha from the Asteraceae family is a weed, commonly termed a mile-a-minute weed. This plant is a perennial creeping weed and grows extremely fast (Li, Li, Li, Wang, & Cao, 2013). This plant is native to Central and South America at the tropical zones but has become widely distributed in Southeast Asia, South China, Pacific Islands, India etc. Several parts of this plant have been used traditionally for disease treatments. Leaves of M. micrantha are applied in case of snake bites or scorpion sting in the form of a poultice and decoction made from the leaves is used to bathe skin itches or rashes. The plant has popularity in Jamaica as it is used for dressing wounds and to promote wound healing (Li, Li, Li, Wang, & Cao, 2013). Recently, some reports have been made from studies of the pharmacological properties of the plant which includes anti-inflammatory activity (Mc, Ocotero, Balcazar, & Jiménez, 2010), antibacterial activity (Haisya, Latifah, Suratno, Sa'diah, & Afiff, 2013; Hajra, Mehta, Pandey, John, & Mehta, 2010; Mc, Ocotero, Balcazar, & Jiménez, 2010), antistress activity (Ittiyavirah & Sajid, 2013), trypanocidal activity (Laurella et al., 2012), antiviral activity (Laurella, et al., 2012), inhibitory effect against plant pathogens (Li, Li, Li, Wang, & Cao, 2013) and antispasmodic effect (Colares, Muguerza, Rosella, & Consolini, 2013). These findings turned the attention of natural product chemists towards the plant. Some major class of plant secondary metabolites

like flavonoids, polyphenols, diterpenes and sesquiterpene lactones have been isolated from the plant.

For this reason, the current investigation was carried out to find possible antidepressant potentials of the methanol extract obtained from the leaves of *Mikania micrantha* in the animal model to predict possible antidepressant action. Moreover, the hypothesis that the leaves of M. micrantha can be used to treat diarrhoea was also aimed to be proved from the findings of present work that involved animal models i.e., Swiss albino mice. Extract from *M. micrantha* also has clot lysis activity which was evident from the study.

2. Materials and Methods

2.1. Plant material

The entire fully matured plant of *Mikania micrantha* was collected from Shitakonda, Kumira, Chittagong and identified by the taxonomist Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

2.2. Preparation of plant extract

Adequate numbers of leaves of plants were air-dried for 10 days. After that, the leaves were placed in an oven to further dry at 45°C for 72 hours. After the completion of drying, the dried sample was milled using a mechanical grinder. The powder thus obtained was kept in an airtight container for further processing. For extraction, 575 gm of the powder was soaked in 2.5 L methanol and the process of shaking was performed for 15 days on a shaker machine and manually as well. Then filtered concentrated extract contained in a beaker was kept in a temperature of 40°C to 50°C range to remove/evaporate the solvent. The weight of the crude extract was 15 g which had a yield of 2.5 % w/w and was stored in the refrigerator.

2.3. Chemicals

Loperamide (Square Pharmaceuticals Ltd., Bangladesh), Castor oil (WELL's Heath Care, Spain), Streptokinase (Beacon Pharmaceuticals Ltd., Mymensingh, Bangladesh), Tween-80 (BDH Chemicals, UK) were procured and used in the experiment. All other chemicals and reagents used in this study were analytical reagent grade.

2.4. Experimental Animals

For this study Swiss albino mice were used as an animal model. The weight range of mice was 25-30 g. Mice were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were provided with standard laboratory food and distilled water and maintained at natural 12 h day-night cycle at 25 ± 2 °C room temperature having proper ventilation in the room

for 10 days in the animal house for acclimation. The protocol followed for this investigation was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

2.5. Antidepressant test

2.5.1. Forced swimming test (FST)

According to the previously established method (Cryan, Valentino, & Lucki, 2005), the forced swimming test was performed to evaluate the antidepressant activity. An open glass chamber of dimensions (25 × 15 × 25 cm) was filled with fresh water to a height of 15 cm and the temperature was maintained at 25° ± 1°C. Then each mouse was forced to swim inside the container. Five groups of mice were utilized and each group contained six mice. Group I received the only vehicle, and Group II was administered with standard drug (diazepam: 5 mg/kg, b.w.; i.p.). Test sample (MEMM 200 mg/kg and 400 mg/kg, b.w.; p.o.) was administered in groups III–IV. A total of 7 minutes of activity was recorded. The initial 2 min was considered for the time required for the mice to adapt to the situation. Then within the later 5 minutes, the duration for which mice remained immobile was recorded. A mouse was considered immobile when it stopped struggling and remained motionless in the water except the movement required to keep its head above the water level.

2.5.2. Tail suspension test (TST)

The time duration of immobile state induced by TST was measured by the method illustrated previously (Wang, Liu, Wang, Lei, Li, & Quan, 2019). Shortly, mice are divided into five individual groups consisting six mice in each group. Vehicle was given in group I, whereas standard medication (diazepam: 5 mg/kg, b.w.; i.p.) was given in group II, and test sample (MEMM 200 mg/kg and 400 mg/kg, b.w.; p.o.) was given in Groups III–IV. Visual and acoustic stimulation was blocked from reaching the test animals while they were suspended by their tail using a tape placed about 1 cm above the tip. The animals were kept 50 cm above the floor. The room was dimly lighted for this experiment and each of the mice was used only once to record the response.

2.6. Castor oil-induced diarrheal test

The method as published previously (Shoba & Thomas, 2001) was used to assess antidiarrheal activity. Five groups of mice were taken for the test. The test animals subjected to 24 hours of fasting before the test. 1% Tween 80 v/v solution was prepared freshly in distilled water. This solution was given to mice group-I and the group was considered as control group. The

antimotility drug loperamide (5 mg/kg, p.o.) was given to group-II of mice and this group served as a positive control group. Methanol extract at a concentration of 200, and 400 mg/kg were administered to mice group-III and group-IV respectively. Tween 80 solution (1% v/v) was used as a solvent for the extract. After administration of the plant extract orally 1 mL of castor oil was given through the same route to each mouse. Each mouse was placed in a cage individually. Blotting papers were placed on the bottom of the case over which the mouse was placed and observed for 4 hours at one-hour intervals. The number and consistency of fecal droppings were noted for each mouse. Blotting papers were changed if required to observe the frequency and nature of stool. The average number of stools produced by the test sample groups was then compared with that of the control group. The average number of stools induced by the action of castor oil in the control group was considered to be 100%. The following equation is used to calculate the level of inhibition (in percentage) of diarrhoeal defecation by the extract administered.

Inhibition of defecation (%) = [(NDC−NDT)/NDC] ×100,

Where NDC = mean number of diarrheic faeces of the control group and NDT = mean number of diarrheic faeces of the treated group.

2.7. Thrombolytic activity test

2.7.1. Streptokinase (SK) Solution Preparation

Lyophilized streptokinase (Polamin Werk GmbH, Herdecke, Germany) of 15,00,000 I.U was thoroughly mixed with sterile distilled water (5 mL). This served as a stock solution from which 30,000 I.U. (100 μL) was taken to perform in vitro thrombolysis test.

2.7.2. Specimen

Five healthy human volunteers were selected and ensured that no one had ever taken oral contraceptives or anticoagulant therapy. From them, 5 mL of blood was taken from each. From each sample obtained 500 μL of blood was taken into five individual alpine tubes which were weighed previously. All tubes were kept stationary to let the blood clot. The same process was followed for every treatment group i.e., positive control and each concentration of extract.

2.7.3. Clot Lysis method

The method for conducting the clot-lysis experiment was reported by Prasad, Kashyap, Deopujari, Purohit, Taori, and Daginawala, in 2006 and was followed here in this study. Blood obtained from the vein from healthy volunteers was kept in a sterile Eppendorf tube (500μL/tube) which were

weighted previously and incubated at 37°C for 45 minutes for the formation of the blood clot. The serum was isolated carefully and completely without disturbing the clot using micropipettes. Then each tube was weighted again to find the weight of the clot blood clot. Then after proper labelling 100 μL of the sample of the extract was added to each tube and the tubes were again incubated for 90 minutes at 37°C. This 90 minutes was to allow the extract to cause lysis of blood clots if there was any capability of the extract to cause such lysis. If there is any clot-lysis activity of the extract the amount of blood clot will be reduced and will lose weight. The fluid thus converted from the blood clot was again removed as was done for the serum previously and the tube was again weighted to find the loss of weighted of clots. Percent of lysis of blood clots was calculated from the weights obtained before and after the lysis of clots. For positive control streptokinase was used as a clot-lytic agent and for negative control distilled water was used. This whole process of the experiment was performed two times changing the volunteers from which blood was obtained. The following equation was used to calculate the percentage of lysis of blood clots by different treatment groups:

% clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100.

This experiment was carried out complying with the Good Clinical Practice (GMP) standards and principles of the declaration of Helsinki (World Medical Association, 2009). The study protocol was reviewed and approved by the ethics committee of the International Islamic University Chittagong, Bangladesh.

3. Results and discussions

3.1. *In-vivo* antidepressant activity

The antidepressant competence of the methanol extract of Mikania micrantha was studied in mice using the forced swimming test (FST). The FST is a rodent behavioral test used for evaluating antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations performed to render or prevent depressive-like states (Can, Dao, Arad, Terrillion, Piantadosi, & Gould, 2012). The result is depicted in Figure1.

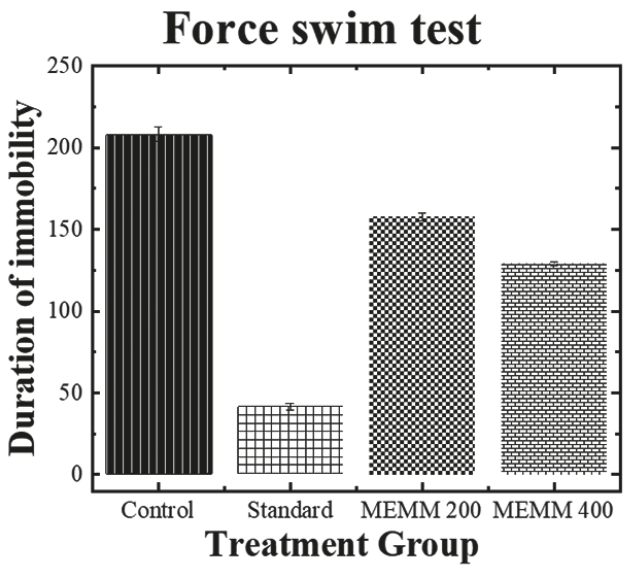

Figure.1
Effect of the methanol extract of Mikania micrantha of antidepression test on force swim test in mice.

Mean values and SEs (N = 6) of duration of immobility are shown as Colum chart.

Fluoxetine-HCl and 1% tween 80 were administered to positive control and negative control groups respectively. Immobility time for the positive control group was measured at 41.56 ± 2.03 seconds and for the negative control group it was comparatively longer i.e., 208.33 ± 4.23 seconds. Observations were found to be in line with the literature reports (Antkiewicz-Michaluk, Wąsik, Możdżeń, Romańska, & Michaluk, 2014). Further notable decrement in the mobility times (157.34 ± 2.08 and 129.67 ± 1.33 seconds) of mice was observed with the administration of the extract MEMM (200 and 400 mg/kg, p.o.), respectively (Figure1.) compared to the negative control group. In general, the MEMM 200 mg/kg and MEMM 400 mg/kg dose of the plant extract displayed enhanced antidepressant activities compared to the control group.

A comparative analysis of the results indicated that the antidepressant potential of MEMM increases in a dose-dependent manner (Figure1.). These observations of the in vivo studies suggest that the permeability to the BBB of MEMM increased with the volume and might be responsible for the augmentation of other pharmacological activities (Mc, Ocotero, Balcazar, & Jiménez, 2010). Based on the literature review, antidepressants can reduce or inhibit oxidative stress related to depressive disorders (Pietta, 2000).

Although it has been identified that plants under investigation have antidepressant activity (Moalem, Hosseinzadeh, & Ghoncheh, 2007), the antidepressant effect of isolated compounds in *Mikania micrantha* needs to be further explored.

3.2. Tail suspension test

The tail-suspension test is an important way of assessing the behavioural activities of the mouse in order to screen the antidepressant potentials of new drugs and to assess other type of manipulations that are supposed to have an important effect on depression-related behaviours (Can, Dao, Arad, Terrillion, Piantadosi, & Gould, 2012). The TST is a well-characterized behavioral model performed to predict antidepressant activity. While performing this test, the test subjects (animals) are kept in a particular condition, and the activity similar to antidepressant drugs, if any, is expressed by the decrease in time in which the subjects remain immobile. This type of effect is shown by conventional antidepressants in this type of experiment (Cryan, Mombereau, & Vassout, 2005; Steru, Chermat, Thierry, & Simon, 1985). Some compounds may show false-positive outcome in the TST which is due to their ability to increase the locomotor activity of test subjects. This study shows that methanol extract of Mikania micrantha (200 and 400 mg/kg, PO) reduced the immobility time (121.67 ± 2.73 and 97.67 ± 2.96 respectively) in Figure 2.

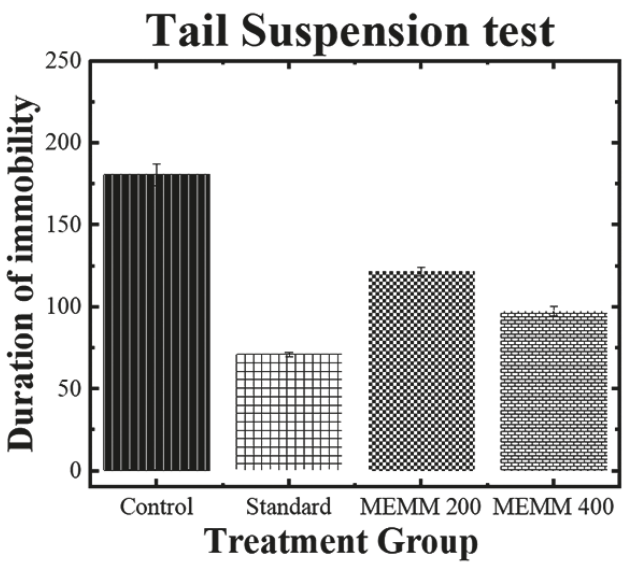

Figure 2

Effect of the methanol extract of Mikania micrantha of antidepression test on tail suspension test in mice.

Mean values and SEs (N = 5) of the duration of immobility are shown in the Colum chart.

Conventional antidepressant drugs can reliably lower the time at which animals remain immobile during the test. If a candidate drug can lower this time of immobile state then it can be reliably predicted that the drug might have antidepressant potential (Porsolt, Bertin, & Jalfre, 1977). Treating the mice groups with the extract for consequtive 7 days exhibited significant antidepressant effects in both methods when compared with the control group which was treated with vehicle only. We found that the anti-immobility result of MEMM in TST and FST exhibited a substantial and dose-dependent effect (Figure2.). Our study demonstrated that MEMM exerts an antidepressant-like impact by employing TST and FST in mice. Hence, MEMM may be studied further in order to find a novel therapeutic way to treat mental depression.

3.3. Castor oil-induced diarrhoea

Castor oil hinders intestinal water reabsorption; in addition, it can change the synchronization of gut motility and thereby foster higher fluid loss (Izzo, Mascolo, Capasso, Germanò, De Pasquale, & Capasso, 1999). Many anti-diarrheal agents work by inhibiting GIT motility (Akah, Aguwa, & Agu, 1999). The anti-diarrheal assay evaluated extracts' effect in retardation of contractibility and movability of gut muscle resulting in the lower intestinal passage. It was found that the extract efficiently represses the onset time and the severity of castor oil-induced diarrhoea (Sharma, Vidyasagar, Singh, Ghule, & Kumar, 2010).

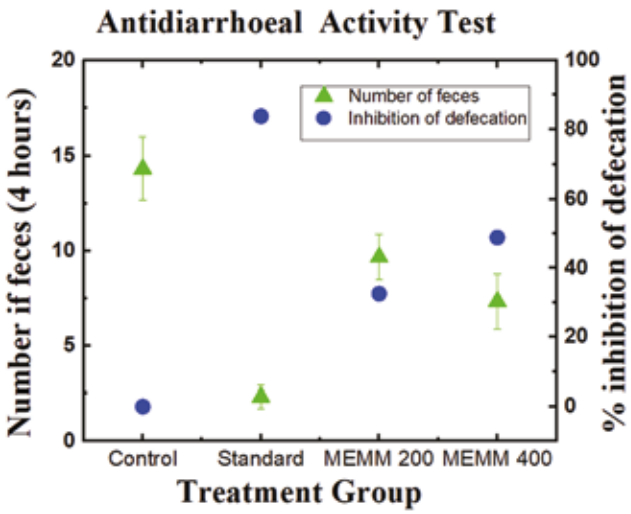

Figure 3

Effect of methanol extract of Mikania micrantha leaves on castor oil-induced diarrhoea in mice.

Dependence of the number of feces and % of inhibition of defecation on treatment group. Mean values and SEs (N = 5) of the number of feces are shown (green Δ). % of inhibition of defecation is also shown (blue ●).

As depicted in Figure3 the diarrhoea was apparent 1 hour after the administration of castor oil in all test animals of the control group for the next 4 hours. Diarrheal episodes were predominantly reduced by the IP injection of loperamide 5 mg/kg (83.81%). The anti-diarrheal effect in the dose of 200 mg/kg (32.52%) & 400 mg/kg (48.85%), so the extract exhibited a significant reduction of diarrhoea over 4 hours. Here, loperamide was used as a positive control group as a contrast to decide whether Mikania micrantha leaf extract showed an antidiarrhoeal effect or not. Water was used as a negative control to identify that is there any antidiarrheal effect was shown by the solvent. These processes were carried out to determine that the calculated antidiarrheal effect was precise and effects have come from Mikania micrantha. Loperamide perform antidiarrheal action by slowing down gastrointestinal motility through the interaction with opioid receptors present in the intestine (Hoan & Rahardja, 2002). Loperamide also causes a long duration of transit time in the intestine. And balance the secretion and absorption of fluid in the mucous membrane of the intestine (Sari, Indriani, & Febrianti, 2018). The test results of two individual doses of methanol extract of Mikania micrantha leaves showed a significant difference in mice. In previous reports, it was suggested that as prebiotic manufacturing, *M. micrantha* had potential antidiarrheal effects by the combination with pouring flour (Harmayani, Aprilia, & Marsono, 2014). Thus, it is desirable to prepare herbal formulation using *Mikania micrantha* leaf extract so that the community people can consume it directly.

3.4. Thrombolytic activity

The blockage of blood flow within blood vessels, hypercoagulation of the blood, and damage to blood vessels result in thrombosis. In this case, diseases such as cerebrovascular ischemia, arthritis, pulmonary embolism, myocardial infarction, venous embolism, and stroke become life-threatening vascular complications. The second most significant cause of cancer-related morbidity is attributed to venous thrombosis (Roy, Jauhari, & Bharadvaja, 2018). The current research was carried out to find out the thrombolytic potential of the methanol extract of Mikania micrantha leaves. A series of studies were performed to find the natural plant sources which consist of thrombolytic (anti-coagulant and anti-platelet) activity. Research suggests that stokes and cardiac disorders may be prevented by the use of such a natural product (Emon, Jahan, & Sayeed, 2020). Various plant sources

showed thrombolytic capacity like Ginger *(Zingiber officinale)*, Garlic *(Allium sativum)*, *Pleurotus ostreatus, Flammulina velutipes, Lumbricus rubellus, Crocus sativus Linn* (Indraceae), *Ganoderma lucidum, Spirodela polyrhiza,* chungkook-jang and natto correspondingly (Emon, Jahan, & Sayeed, 2020). The percentage of lysis of blood clots obtained after subjecting clots with different sample concentrations has been outlined in Table I.

Table I
Thrombolytic Activity of Mikania micrantha compared with Streptokinase

Treatment	% of clot lysis
Water	5.07 ± 0.12
Mikania micrantha (200)	25.39 ± 4.98
Mikania micrantha (400)	43.76 ± 3.24
Streptokinase	76.54 ± 0.09

The in vitro thrombolytic activity study revealed that the methanol extract of Mikania micrantha has appreciable clot lysis activity. The percentage of loss of weight from clots after applying the extract was considered as the functional indication of possible thrombolytic potential.

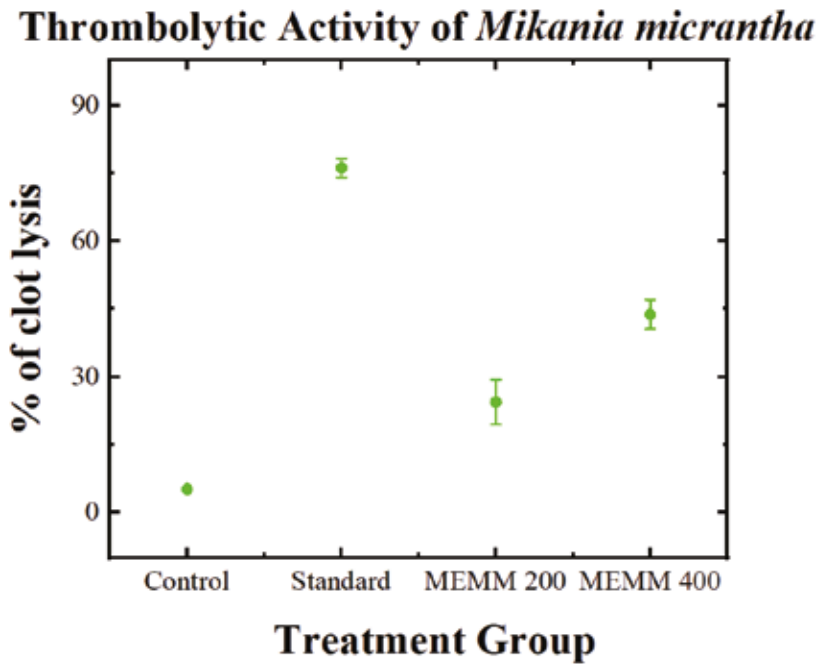

Figure 4.
Thrombolytic activity of Methanol extract of Mikania micrantha, Streptokinase & Water.

Mean values and SEs (N = 3) of % of clot lysis are shown (green ○).
The thrombolytic activity was determined through two concentrations of extract and compared with the standard. The lysis of clot for extract (200 mg) is 25.39.15% and for extract (400 mg) is 43.76 % whereas the clot lysis for standard Streptokinase (30,000 IU) is 76.54 ± 0.09%.and the lysis by the control was negligible at 5 ± 0.12 % [Figure 4]. Since the extract solution showed moderate clot lysis compared with the standard, it was noticed that the extract has potent thrombolytic activity, which is supported by the findings from a previous study on this plant (Raka, Mishu, Rahman, & Rahman, 2019). As in most cases, thrombolytic agents remove the fibrin through the interaction with the plasminogen enzyme which results in optimal blood supply in blood-congested narrow vessels (Bhattacharya, Ploplis, & Castellino, 2012). The clot lysis activity observed for the MEMM means that methanol soluble compounds of *Mikania Micrantha* contain thrombolytic responsible substances (Emran et al., 2015). To determine the active substance(s) that may provide a new arena to find out a safe drug in treating cardiovascular disease, further researches are required.

4. Conclusion

The overall results of the current studies indicated that extracts of *M. micrantha* leaves can be a noticeable source for significant neuro-pharmacological as well as antidiarrheal and thrombolytic activities. In addition, it can be considered in the treatment of anxiety and depression. However, additional studies are also recommended to identify the components followed by the isolation and characterization of Phyto-constituents which are responsible for these pharmacological bioactivities and to establish the mechanism of action of such activities for better therapeutic importance. Further investigation of the different plant parts of *M. micrantha* is strongly recommended.

Ethical Approval

According to the Helsinki Declaration 2013 regarding ethical guidelines, this study conformed to those rules and regulations during every screening of biological activities. Guidelines related to animal euthanasia in 2013 version were followed and all animals were euthanized according to the Swiss Academy of Medical Sciences and the Swiss Academy of Science.

Declaration of competing interest

The author declares no financial interests.

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