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Comparative valorisation on biological activities of *Leea indica, Oroxylum indicum, Mikania micrantha* and *Smilax macrophylla* as a sustainable source of bioactive ingredients

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Abstract

In Bangladesh, *Leea indica, Oroxylum indicum, Mikania micrantha and Smilax macrophylla* have been reported to have medicinal properties to treat numerous types of diseases, such as inflammation, pain, diarrhoea, skin problems and asthma. The present study designed in a comparative manner, assesses the qualitative phytochemical analysis, cytotoxic and antinociceptive activities of methanol leaves extract of *L. indica* (MELI), *O. indicum* (MEOI), *M. micrantha* (MEMM), and methanol extract of *S. macrophylla* leaves (MESMl) and stems (MESMs). The methanol extract of each plant comprises a significant quantity of secondary metabolites in the phytochemical study. At the same time, a non-significant weak to high toxicity in the brine shrimp lethality bioassay was assessed. Acetic acid and formalin-induced antinociceptive activity demonstrated highly significant ($p < 0.001$) dose-dependent pain reduction at a higher dose (400 mg/kg). Hence, current findings suggest that all five methanol extracts have potential antinociception effects along with weak to high cytotoxicity. Remarkably, these plants can be a successful substitute besides agri-food substances in the treatment of nociception.

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1. Introduction

The acceptance and demand for medicinal plants are increasing day by day (Singh, 2002) since they are used to treat human disorders (Firenzuoli & Gori, 2007). According to WHO, traditional medicinal plants are natural plant products used to treat local or regional disorders in the absence of industrial products (Tilburt & Kaptchuk, 2008). Almost 80% of the world's

population depends on traditional plant medicines, acting as primary health aid (Veiga et al., 2020). Currently, chemotherapy is one of the most frequently used methods to treat cancer that associates toxic side effects (Rafieian-Kopaie & Nasri, 2015). Therefore, screening the raw extracts of natural plants for cytotoxic activities is necessary to find safer alternatives (Rafieian-Kopaie & Nasri, 2015).

Many diseases and wounds are marked by cancer and pain (Banu et al., 2020; Goudas et al., 2005), but also by pain and inflammation with different physiological actions may grow individually. Nociception is the process of triggering factual tissue damage is transmitted to the brain in the spin thalamic tract, which processes and interprets the information and activates the descending modulation system of pain (Kurita et al., 2019). It is a warning sign and leads to numerous unfavorable impacts (Hasan et al., 2010). Analgesics act by blocking the pain signalling pathway of the central nervous system (CNS) (Korni et al., 2020). Notable medications like- opiates and NSAIDs used for nociception are derived from natural products. Other synthetic compounds are evaluated, which act by the same mechanism, while they have several major adverseeffects such as nausea, ulceration, gastrointestinal (GI) bleeding, respiratory illness, sleepiness, etc. (Mate et al., 2008). Besides, natural-products have a significant role in the discovery and development of pain-relieving or analgesic-drugs with the mechanisms of pain transmission and relief from pain (Hasanat et al., 2019). Several plant-derived compounds could offer important therapeutic impacts for interminable inflammatory disorders, which are likely to connect with pain. Right now, numerous formulas are available to treat pain; among them, therapeutic spices are featured as well known for their use and convenience (Abdel-Rahman, 2017).

Moreover, recent epidemiological studies have shown a global increase in the incidence and prevalence of cancer, particularly in the elder patient (Caraceni & Shkodra, 2019). More than 50% of cancer patients are killed, with 90% suffering from pain. In order to provide a decent quality of life for terminally ill people, effective pain control medication is necessary. So, there is a quest for bioactive compounds, which could be a potential source to treat cancer and analgesia with fewer side effects (Rahman et al., 2020). In this study, we explored the possible cytotoxic and antinociceptive activities of four traditionally used medicinal plants- *Leea indica, Oroxylum indicum*, *Mikania micrantha*, and *Smilax macrophylla*. *Leea indica* (Family: Leeaceae) is traditionally used in the treatment of headache, body pain, skin problems (Bais, 2013), diarrhoea, spasm, and shigellosis. Several bioactive compounds such as- **β**-sitosterol, palmitic acid,1-eicosanol, solanesol, farnesol, phthalic acid

esters, gallic acid, lupeol and ursolic acid were reported from its leaves (Srinivasan et al., 2008). *L. indica* possesses severalpharmacological properties such as: cytotoxic, antimicrobial, antioxidant, analgesic, thrombolytic, antitumor, and antidepressant activities (Mishra et al., 2016). *Oroxylum indicum* (Family: Bignoniaceae) is reported to have several essential oils and flavonoids derived compounds (Siddiqui et al., 2012); along with chrysin, oroxylin-A, scutellarin, baicalein, quercetin-3-o-α-L-arabinopyranoside, 1-(2-hydroxyethyl) cyclohexane-1, 4-diol and apigeninisolated from its leaves (Yuan et al., 2008). Traditionally it is used against fever, diarrhoea, shigellosis, respiratory illness, intestinal parasites, skin disorder or hypopigmentation, asthma, inflammation, constipation, etc. The fruits and seeds are used in bitter tonic, purgative, cough syrup (Bais, 2013). It also possesses antibacterial, antioxidant, anti-inflammatory, analgesic, hepatoprotective, antidiabetic, neuroprotective, anthelmintic, immunomodulatory and anticancer pharmacological activities (Siddiqui et al., 2012). *Mikania micrantha* (Family: Asteraceae) is applied in folk medicine with several uses against insect bites, skin disorders (rashes, itches) (Li et al., 2013). Traditionally it is useful in external bleeding while reported to have antimicrobial activity (Nurdiana et al., 2013). Six compounds were isolated from the leaves of *M. micrantha,* namely, deoxymikanolide, scandenolide, dihydroscandenolide, mikanolide, dihydromikanolide, and *m*-methoxy benzoic acid (Li et al., 2013). *Smilax macrophylla* (Family: Smilacaceae) is used in traditional Chinese-medicine for detoxification, elimination of damp, remove wind evil (Afifi & Kasabri, 2013). It is also useful in treating fever, skin and reproduction disorder, gout, inflammation of the eye, diuretic, to treat infertility (Chhabra et al., 1993). It has several isolated bioactive compounds such as 3-methyl-1-pentene; 2-nitrobutane; 3, 4-dimethylpentane; 2, 2-dimethylpentane; 2, 4-dimethylpentane; 2, 2, 3-trimethylbutane; 2, 3-dimethylpentane; 2, 4-dimethylhexane; 2, 3, 3-trimethylbutane; 3, 4-dimethylhexane; 1, 2, 4-trimethylcyclopentane; 2, 2, 3, 4-tetramethylpentane; 2, 4, 6-trimethylheptane; 3-ethyl-2,5-dimethylhexane; 2,6-dimethyloctane; n-decane; alpha-tocopherol-beta-d-mannoside; n-hexatriacontane, etc. (Zubair et al., 2017). There is a report about pharmacological activity of anticancer, antidiabetic, skin disorder, anti-inflammatory, ulceration, and antioxidant for *S. macrophylla* (Damayanthi et al., 2011). The present study was planned toinvestigate and compare bioactivities of various plants in vitro and in vivo models. It is the first the comparative investigation of cytotoxic and nociceptive activities among *Leea indica, Oroxylum indicum, Mikania micrantha* and *Smilax macrophylla* leaves and stems. This research substantially aimed to validate these by-products

extracts for cancer, pain and inflammation applications as biologically active substances. Thus, the present research focuses on the extraction of the plants in order to study of the cytotoxic and antinociceptive activity with their qualitative phytochemicals.

2. Materials and methods

2.1. Chemicals and reagents

Diclofenac sodium was purchased from Beximco Pharmaceuticals Ltd., Bangladesh, non-ionized NaCl sea salt, vincristine sulfate (Sigma-Aldrich Co), formalin (Merck, Mumbai, India), acetic acid (Merck, Mumbai, India), normal saline solution (0.9% NaCl) and Tween-80 (BDH Chemicals, UK) and other chemicals were purchased from local supplier Taj Scientific, Ltd. (Chittagong, Bangladesh). The entire chemicals used in this experiment were of analytical grade.

2.2. Preparation of plant extracts

L. indica, O. indicum, M. micrantha leaves and *S. macrophylla* leaves and stems were collected from Kaptai, Rangamati Chittagong (22°30'N 92°13'E), leaves and stems were dried and minced into a coarse powder. The grounded powder was macerated with methanol, kept in a water bath (45 °C) for evaporation, and followed for filtration. The maceration of powder (g) and methanol solvent (mL) was mixed in 1:4 ratios. The filtration was followed by the Whatman-filter paper (#1). After filtration, a semi-solid extract was obtained, which was stored at the refrigerator for further use (Prawej et al., 2017).

2.3. Experimental animals

For this study, male and female Swiss-albino mice were obtained from Jahangirnagar University, Savar, Bangladesh. The animals were housed in polypropylene cages (as two pairs of mice per cage). By supplying normal laboratory food and distilled water ad libitum, where the animals were familiarized with the laboratory conditions over 10 days (12h dark/12h light cycle; temperature 25 ± 2 °C). This research work was carried out under the guidelines of ARRIVE(Animal Research: Reporting of in vivo experiments) and approved by the Committee of Planning and Development (P&D), Department of Pharmacy, International Islamic University Chittagong, Bangladesh (Pharm-107/11-17).

2.4. Qualitative phytochemical analysis

The methanol leaves extract of *L. indica* (MELI), *O. indicum* (MEOI), *M. micrantha* (MEMM), and methanol extract of *S. macrophylla* leaves (MESMl)

and stems (MESMs) were tested for the phytochemical screening by standard procedures to evaluate the alkaloids, carbohydrates, flavonoids, terpenoids, tannins, saponins, polyphenols, quinines, fixed oils, cardiac glycosides, fatty acids, phlobatannins, coumarins, oxalates, proteins, resins, cholesterols, gums and mucilages, phytosterols and sterols (Auwal et al., 2014; Banu & Cathrine, 2015; Hossain et al., 2013; Ugochukwu et al., 2013). A brief description of the qualitative phytochemical methods was presented in the Supplementary section.

2.5. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was screened for cytotoxic activity (Meyer et al., 1982). The driedcysts of shrimp were hatched in a 3.8% NaCl solution (artificial seawater) for 48 hours. The MELI, MEOI, MEMM, MESMl, and MESMs were mixed with dimethyl sulfoxide (DMSO) in a concentration of 50 µL in 5 mL. Then, mixed with the artificial seawater of 3.8% NaCl solution to get a serially diluted concentration at 10, 50, 100, 200, 300, 400, 500, 800, 1000 µg/mL. Each test tube contained ten nauplii and the test was performed in a triplicate manner. The numbers of alive nauplii were counted after 24 hr by using a magnifying glass. A series of concentration dilutions at 0.125, 0.25, 0.5, 1, 2.5, 5, 10, 20 and 40 µg/mL were prepared for positive control with vincristine sulfate. The LC_{50} or lethal concentration was calculated from the regression equation. The percentage of mortality was calculated using the following equation:

$$\% \text{ Mortality} = \frac{N_O - N_1}{N_O} \times 100 \quad (1)$$

Where, N_0 = Number of nauplii taken, and N_1 = Number of nauplii alive. In general, the higher the value of the LC_{50}, the lower the toxicity and vice versa. The LC_{50} >1000 µg/mL value is non-toxic, weakly toxic (500 - 1000 µg/mL), moderately toxic (100 - 500 µg/mL), and is considered to be highly toxic (LC_{50}<100 µg/mL) (Nguta, Mbaria, Gathumbi, et al., 2011).

2.6. Antinociceptive activity

2.6.1. Acetic acid-induced writhing test

The antinociceptive activity was evaluated by the acetic acid-induced writhing test. Here, MELI, MEOI, MEMM, MESMl and MESMs (200 and 400 mg/kg, b.w.) were treated orally by using gavage, diclofenac sodium (10 mg/kg, b. w. i. p.) as the reference drug, and 1% Tween-80 for negative control (10 mL/kg, b. w.). Thirty minutes later, 0.6% (v/v) (10 mL/kg b. w.) acetic acid was induced intraperitoneally in each group of mice (n=3). The

experiment was monitored for 20 minutes, whereas the number of writhing was counted and recorded (Adnan et al., 2020). The percentage (%) of inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{Nc - N_S}{Nc} \times 100 \quad (2)$$

Where, N_c = Number of writhing by control, and N_s = Number of writhing by drug/extract.

2.6.2. Formalin Induced Licking Test

Formalin induced licking test was screened according to Burgoset al. (Burgos et al., 2010). MELI, MEOI, MEMM, MESMl and MESMs (200 and 400 mg/kg, b. w.) were treated orally by using gavage, diclofenac sodium (10 mg/kg, b.w., i.p.) as reference drug and 1% Tween-80 for negative control (10 mL/kg, b.w.). After 30 minutes, a solution of 20 µL formalin (2.5%) was administrated in the right hind paw (sub-plantar region), and the time spent in licking and biting was counted. The observation for licking and biting was relative to the total licking time. Two phases were represented: the early phase (0-5 min) and the late phase (15-30 min). The following equation was used to calculate the % of inhibition:

$$\text{Inhibition (\%)} = \frac{Nc - N_S}{Nc} \times 100 \quad (3)$$

Where, N_c = Number of licking time by control, and N_s = Number of licking time by drug/extract.

2.7. Statistical analysis

Values were represented as Mean ± standard error mean (SEM) (n = 3). One-way ANOVA (Dunnett's test) for statistical analysis (SPSS 16.0, IBM Corporation, NY) was used, where $p < 0.05$, $p < 0.01$ and $p < 0.001$ consider statistically significant compared with the negative control group.

3. Results

3.1. Phytochemical analysis

The phytochemical constituents of MELI, MEOI, MEMM, MESMl, and MESMs were summarized in Table I**.** Here, alkaloids, carbohydrates, tannins and proteins are present in all extracts, whereas the flavonoids and quinones are only present in MEOI and MELI.

Table I
Comparative phytochemical screening of MELI, MEOI, MEMM, MESMl and MESMs.

Sl No.	Test Name	MELI	MEOI	MEMM	MESMl	MESMs
1.	Alkaloids	+	+	+	+	+
2.	Carbohydrates	+	+	+	+	+
3.	Flavonoids	+	+	-	-	-
4.	Terpenoids	-	-	-	-	-
5.	Tannins	+	+	+	+	+
6.	Saponins	-	-	-	-	-
7.	Polyphenols	+	-	-	-	-
8.	Quinones	+	+	-	-	-
9.	Fixed oils	+	-	+	+	+
10.	Cardiac glycosides	+	-	+	+	+
11.	Fatty acids	-	-	-	-	-
12.	Phlobatannins	+	+	-	-	+
13.	Coumarins	-	-	+	+	+
14.	Oxalates	-	-	-	-	+
15.	Proteins	+	+	+	+	+
16.	Resins	+	-	-	-	-
17.	Cholesterols	-	-	-	-	-
18.	Gums and mucilages	-	-	-	-	-
19.	Phytosterols	-	-	+	-	-
20.	Sterols	-	-	-	-	-

- = Absent, + = present.

MELI- methanol extract of *Leea indica*; MEOI- methanol extract *Oroxylum indicum*; MEMM- methanol extract of *Mikania micrantha*; MESMl- methanol extract of *Smilax macrophylla* leaves and MESMs- methanol extract of *Smilax macrophylla* stem.

3.2. Brine shrimp lethality bioassay

The percentage of mortality with the LC_{50} value and regression equation was summarized in Figure 1. A non-significant percentage of mortality was exhibited by the extracts. The LC_{50} of MESMs (503.776 μg/mL) was found to be weakly toxic, while MEOI (373.38 μg/mL), MEMM (262.05 μg/mL), and MESMl (468.44 μg/mL) were found to be moderately toxic. On the other hand, MELI (36.01 μg/mL) was found to be highly toxic, while LC_{50} was 6.15 μg/mL for vincristine sulfate acted as standard.

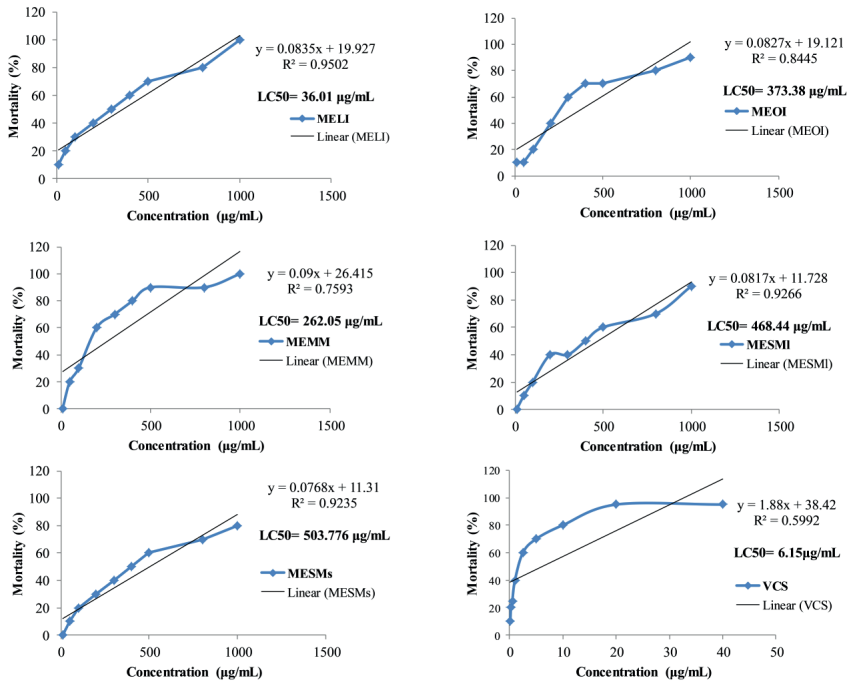

Figure 1

Percentage of mortality of MELI, MEOI, MEMM, MESMl and MESMs with LC50 and regression value. MELI- methanol extract of Leea indica; MEOI- methanol extract Oroxylum indicum; MEMM- methanol extract Mikania micrantha; MESMl- methanol extract Smilax macrophylla leaves; MESMs- Methanol extract of Smilax macrophylla stem and VCS- Vincristine sulfate.

3.3. Antinociceptive activity

3.3.1. Acetic acid induced writhing test

All the extracts showed a dose-dependent significant inhibition of nociception. The MEMM (200 and 400 mg/kg) showed a significant ($p < 0.001$) dose-dependent percentage of inhibition of nociception (43.19 and 64.31%), while reference drug of diazepam exhibited 75.12% significant inhibition. The 400 mg/kg of MEOI and MELI exhibited between 25.82 and 40.38% significant ($p < 0.001$) inhibition of nociception. Observations of the test were summarised in Table II.

Table II
Acetic acid induced writhing test of MELI, MEOI, MEMM, MESMl and MESMs.

Treatment	No. of Writhing	Inhibition (%)
Control	71.0 ± 2.65	–
Diclofenac Sodium (10 mg/kg)	17.67 ± 1.20 [c]	75.12
MELI (200 mg/kg)	62.33 ± 1.45 [a]	12.21
MELI (400 mg/kg)	42.33 ± 1.45 [c]	40.38
MEOI (200 mg/kg)	66.0 ± 5.57	7.04
MEOI (400 mg/kg)	52.67 ± 0.33 [c]	25.82
MEMM (200 mg/kg)	40.33 ± 0.33 [c]	43.19
MEMM (400 mg/kg)	25.33 ± 0.33 [c]	64.31
MESMl (200 mg/kg)	64.33 ± 0.67	9.38
MESMl (400 mg/kg)	50.33 ± 0.33 [c]	29.10
MESMs (200 mg/kg)	66.33 ± 0.67	6.57
MESMs (400 mg/kg)	54.67 ± 0.67 [c]	23.0

Values represented as Mean ± SEM (n = 3). [a] $p < 0.05$ and [c] $p < 0.001$ was considered statistically significant (Dunnett's test). MELI- methanol extract of *Leea indica*; MEOI- methanol extract *Oroxylum indicum*; MEMM- **methanol extract** *Mikania micrantha*; MESMl- methanol extract of *Smilax macrophylla* leaves and MESMs- methanol extract of *Smilax macrophylla* stem.

3.3.2. Formalin induced licking test

Formalin induced licking test for analgesic activity was summarized in Table III. All extracts demonstrated a significant percentage of inhibition in both phases compared with the control group. The MEMM extract at 200 and 400 mg/kg exhibited maximum inhibition of nociception in a dose-dependent manner: in the early phase (46.28 and 62.81%, $p < 0.001$) and late phase (28.33 and 47.03%, $p < 0.001$). The MELI also revealed dose-dependent manner inhibition of nociception, whereas the reference drug diclofenac sodium confirmed significant inhibition of formalin-induced licking activity in the early phase (78.10%, $p < 0.001$), and late phases (73.94%, $p < 0.001$).

Table III
The effects in formalin test in mice by MELI, MEOI, MEMM, MESMl, and MESMs.

Treatment	Early Phase (0-5 min)	Inhibition (%)	Late Phase (15-30 min)	Inhibition (%)
Control (1% Tween)	80.67 ± 0.36	--	117.67 ± 1.45	--
Diclofenac sodium	17.67 ± 0.41[c]	78.10	30.67 ± 0.67 [c]	73.94
MELI (200 mg/kg)	42.67 ± 0.53 [c]	47.11	92.33 ± 1.45 [c]	21.53
MELI (400 mg/kg)	35.0 ± 0.58 [c]	56.61	62.33 ± 1.45 [c]	47.03
MEOI (200 mg/kg)	56.0 ± 1.0 [c]	30.58	111 ± 0.58 [b]	5.67
MEOI (400 mg/kg)	38.33 ± 0.48 [c]	52.48	73.67 ± 1.20 [c]	37.39
MEMM (200 mg/kg)	43.33 ± 0.41 [c]	46.28	84.33 ± 0.67 [c]	28.33
MEMM (400 mg/kg)	30.0 ± 0.33 [c]	62.81	62.33 ± 1.45 [c]	47.03
MESMl (200 mg/kg)	77.33 ± 0.85	4.13	116.0 ± 1.0	1.42
MESMl (400 mg/kg)	60.33 ± 0.25 [c]	25.21	87.33 ± 1.45 [c]	25.78
MESMs (200 mg/kg)	64.0 ± 0.63 [c]	20.66	92.33 ± 1.20 [c]	21.53
MESMs (400mg/kg)	44.67 ± 0.25 [c]	44.63	74.0 ± 2.08 [c]	37.11

Values represented as Mean ± SEM (n = 3). [b] $p < 0.01$ and [c] $p < 0.001$ was considered statistically significant (Dunnett's test). MELI- methanol extract of *Leea indica*; MEOI-methanol extract *Oroxylum indicum*; MEMM- methanol extract *Mikania micrantha*; MESMl-methanol extract of *Smilax macrophylla* leaves and MESMs- Methanol extract of *Smilax macrophylla* stem.

4. Discussion

Plant-based medicinal products have shown high potential due to their great role in the modern world as safer, cheaper and more efficient medicine (Jordan et al., 2010). Medicines are derived from limitless resources that are pharmacologically active ingredients to manage several diseases (Nissen, 2010). Physiological activities of plant extracts were revealed by phytochemical analysis. Meanwhile, the study of methanol plant extracts shows the presence of alkaloids, carbohydrates, flavonoids, tannins, fixed oils, cardiac glycosides, and proteins. However, the previous acute toxicity analysis has described no mortality and abnormal behaviour of the plant and neurological changes of up to 2000 mg / kg, indicating a low toxicity profile of the plant extract and a safe therapeutic dose (Wong et al., 2012).

The presence of alkaloids in plant extracts is often associated with cytotoxic and analgesic properties (Barua et al., 2020; Yang et al., 2020). Flavonoids are a major group of phytochemicals used for cancer, cardiovascular and anti-inflammatory conditions (Bertleff-Zieschang et al., 2017; Sampaio et al., 2016). A low concentration was recommended to be tested to measure the potency of extracts (Rahman et al., 2013). The percentage of mortality in the cytotoxicity study of methanol extracts showed a non-significant weak to high toxicity. The MELI exhibited 36.01 µg/mL lethality, which is highly toxic, while the MESMs were weakly toxic (503.776 µg/mL). This is often shown as plant extract contains polyphenols that support cell proliferation at a lower concentrations while negative impacts for higher concentrations (Sowa et al., 2020). Besides, the MEOI, MEMM, and MESMl were moderately toxic (373.38, 262.05 and 468.44 µg/mL, respectively) (Nguta, Mbaria, Gakuya, et al., 2011) due to the presence of alkaloids in the extract which requires further analysis to evaluate the mechanism of cytotoxicity (Barua et al., 2020; Guha et al., 2020; Yang et al., 2020). This cytotoxic effect is caused by alkaloids, flavonoids, quinones and tannins of the test extracts had been reported by many other scholars (Gomes et al., 2018; Hosseini et al., 2019; Ivănescu et al., 2018). In comparison to the secondary metabolites of cardiac glycosides may be useful for the antithrombotic effect, which had been exhibited by Ambrosy et. al. (2014). Moreover, plant resins are used in response to injury and to protect against diseases and insects. Therefore, these plant-derived products and

their components can be responsible to block the inception of precancerous cells into malignant ones or reverse promotion, and stop or delay the development (Thun et al., 2004).

Meanwhile, the results of the anti-nociception were evaluated by acetic acid and formalin-induced tests to assess the narcotics and non-narcotics drugs (Bhuiyan et al., 2020). The acetic acid-induced writhing test is not a selective pain test but is highly sensitive. The formalin-induced licking test is sensitive to NSAIDs and other analgesics (Hunskaar & Hole, 1987). The acetic acid-induced intraperitoneal (IP) produces abdominal contraction due to the production of histamine, serotonin, and cyclooxygenase (COX) (Ikeda et al., 2001). Arachidonic acid and prostaglandin biogenesis may affect the nociceptive mechanism by the visceral pain model (Kuehl & Egan, 1980). These types of responses are caused by sensing in ion channels and the prostaglandin by releasing mast cell from peritoneum (Kaushik et al., 2012). Local peritoneal receptors are proposed to be relatively tangled in the response of abdominal constriction (Bentley et al., 1981), whereas it has been connected with the elevation of peritoneal fluid levels in prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) along with the increased levels of lipoxygenase enzymes after inducing acetic acid intraperitoneally (Ayanwuyi et al., 2010; Bhuiyan et al., 2020). Besides, Jiang *et al.* also identified that induction of the acetic acid intraperitoneally elevates the lipoxygenase enzyme level in peritoneal fluid (Jiang et al., 2014). Thus this enzyme may be activated peripheral analgesic mechanism and involving the release of mediators which suppress the analgesia (Bhuiyan et al., 2020). Notably, the crude methanol extract displayed a remarkable ($p < 0.001$) inhibition of writhing in a dose-dependent manner. The presence of secondary metabolites (alkaloids, tannins, coumarins) (Adnan et al., 2019; Bovell-Benjamin & Roberts, 2016; Mohammed et al., 2014) in MEMM (200, 400 mg/kg) may be responsible for showing the maximum percentage of inhibition of nociception (43.19 and 64.31%), respectively, whereas the reference drug diclofenac sodium result was 75.12%. However, plant-derived flavonoids are responsible for increasing the ability of antioxidants and for treating local and systemic diseases, such as celiac disease (Ferretti et al., 2012), cancer (Romagnolo & Selmin, 2012), diabetes mellitus (Babu et al., 2013), cardiovascular disorders (Xu et al., 2014), etc. A higher dose (400 mg/kg) with secondary pharmacologically active metabolites (alkaloids, tannins and coumarins) (Adnan et al., 2019; Bovell-Benjamin & Roberts, 2016; Mohammed et al., 2014) showing maximum nociception decline in MELI (40.38%), MESMl (29.10%), MEOI (25.82%) and MESMs (23.0%). Flavonoids (Calixto et al., 2000) in MELI, MEOI and coumarins

(Bovell-Benjamin & Roberts, 2016) in MESMl, MESMs may also be responsible for the significant antinociceptive effects. The following hierarchy was shown to reduce nociceptive effects: MEMM > MELI > MESMl > MEOI > MESMs. In formalin-induced tests, mice response by licking the hind paw is an indication of analgesia (Hunskaar & Hole, 1987). The formalin-induced licking test in hind paw gives clear evidence in determining the moderate to continuous pain in rodent animals (Chandran et al., 2020), whereas the test was measure in two phases, which reflect different kinds of pain. Here, the early phase is non-inflammatory pain (nociceptors) and the late phase is for inflammation (Hunskaar & Hole, 1987). After inducing formalin, substance P (SP), and bradykinin released in the early phase, whereas inflammatory mediators (histamine, prostaglandins, 5-hydroxytryptamine) is involved in the late phase (Xu et al., 2014; Zhang et al., 2015). In this study, all the extracts exhibited significant inhibition in the early phase, while the late phase also showed a significant dose-dependent manner inhibition of nociception. Like the acetic acid test, MEMM exhibited a maximum pain reduction. The ranking order of reduction of pain for antinociceptive effects is shown below: MEMM > MELI > MESMl > MEOI > MESMs. The effects of MEMM (200, 400 mg/kg) in the early phase were 46.28 and 62.81%, while the late phase depleted into 28.33 and 47.03% inhibition of licking time, whereas the reference drug exhibited similar manner activity in both phases. The results showed significant inhibition in nociception, which may be due to the presence of alkaloids and coumarins (Adnan et al., 2019; Bovell-Benjamin & Roberts, 2016; Mohammed et al., 2014), while the tannins may be responsible for the anti-inflammatory activity of medicinal plants (Dutta et al., 2020; Mohammed et al., 2014). Besides, quinones act as tumor suppressor, inhibition of PGE2 biosynthesis and CVS diseases (Liu, 2011). Our findings reported that the both phases were reduced by this five extract in comparison with the control group, a similarity to Onasanwo and Elegbe observation (Onasanwo & Elegbe, 2006). In the early and late phases of formalin and acetic acid-induced test revealed a harmony and produced a potential inhibition in mice model that act as central and peripheral anti-nociception.

Therefore, this comparative research led to the characterization of the MELI, MEOI, MEMM, MESML and MESM are active secondary components and the empirical evaluation of bio-active purposes. This could justify the ethnopharmacological uses in body pain and inflammation (Bais, 2013). Meanwhile, it is worth remembering the drawbacks of this report. To date, a question has also been raised regarding the suitability of antinociceptives and cytotoxic for intermittent therapy. These studies have

carried out minimal studies on animals to obtain clear evidence of phyto-constituents.

5. Conclusions

The current comparative scientific research finding reflects the existence of secondary metabolites – namely alkaloids, tannins, carbohydrates, glycosides and flavonoids — that the element of the plant possesses biologically active potential compounds with cytotoxic and analgesic activities. It suggests that the methanol extracts of the plants used in this study have analgesic efficacy in the higher dose, but low to high cytotoxicity. The findings of *in vivo* studies indicate that the five extracts have central and peripheral antinociceptive effects allowing future efforts to expand the research and conduct sufficient chromatographic analysis to determine possible secondary metabolites responsible for the above activities. As plant extracts have reported several secondary metabolites, they can be used as a sustainable natural source of bioactive compounds for potential value in the field of agri-food substances and pharmaceutical industry, which can contribute to the economy. Further *in vivo* and *in vitro* studies are required to identify new bioactive compounds from these extracts.

List of Abbreviations: MELI- methanol extract of *Leea indic*a; MEOI- methanol extract of *Oroxylum indicum*; MEMM- methanol extract of *Mikania micrantha*; MESMl- methanol extract of *Smilax macrophylla* leaves; MESMs- methanol extract of *Smilax macrophylla* stems; SEM- Standard error mean.

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List of Abbreviations: MELI- methanol extract of *Leea indic*a; MEOI- methanol extract of *Oroxylum indicum*; MEMM- methanol extract of *Mikania micrantha*; MESMl- methanol extract of *Smilax macrophylla* leaves; MESMs- Methanol extract of *Smilax macrophylla* stems; SEM- Standard error mean; SPSS- Statistical Package for Social Sciences.

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