INTRODUCTION

Depression is an affective disorder characterized by a change in mood, lack of confidence, lack of interest in surroundings and the severity may range from mild to severe [1]. About 450 million people suffer from mental or behavioral disorders. Depression will become the second leading cause of premature death or disability worldwide by the year 2020.A large percentage of patients suffering from depression respond to the currently available antidepressants, but the extent of improvement is still disappointing because of various side effects and drug tolerance [2]. Till today, there are many unmet clinical needs with respect to the efficacy and adverse effects of the various antidepressants. To address these needs, antidepressants with novel mechanisms and fewer side effects are in great demand [3].

It is a fact that many natural products and synthetically modified natural product derivatives have been successfully developed for clinical use to treat human diseases in almost all therapeutic domains [4]. The treatment and control of diseases by using locally available medicinal plants will continue to play significant roles in medical healthcare implementation in developing countries [5].

Globally herbal medicines are extensively used due to their therapeutic efficiency and minimum side effects in neurological disorders; therefore, investigations for the search of novel and better-tolerated molecules from plant sources have progressed in recent years demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [6]. However, to the best of our knowledge, there is no single scientific report demonstrating the antidepressant activity of Sapindus marginatus and Acorus calamus. Current investigation aimed at evaluating the antidepressant potential of Sapindus marginatus and Acorus calamus. Depression represents one of the major health problems among other mood disorders worldwide. The use of suitable animal models is essential for understanding of the neurobiological basis of mood disorders and is facilitating the approaches for the discovery of novel therapeutic targets. Forced Swim Test (FST) and Tail Suspension Test (TST) are employed as exemplary systems to probe depressive condition in rodents [7].

Immobility or despair behaviour produced in both FST and TST were hypothesized to display animal's hopelessness and low mood (behavioural despair), and are taken as paradigm of depression. This simple behavioural procedure is a widely used test for screening novel antidepressants [7]. There are number of pharmacological studies on Sapindus marginatus and Acorus calamus in the literature. Some studies reported the potentials of the two plants as anticonvulsants. Depression is a mood disorder often accompanied with convulsive disorders in humans. Therefore, we also focused on the evaluation and comparison of the effects of the two plants on depression models.

MATERIALS AND METHODS

Approval of institutional animal ethics committee (IAEC)

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, after getting approval of the Institutional Animal Ethics committee, RIMS, Imphal (No.1596/GO/a/12/CPSEA).

Requirements

Albino mice, polypropylene cages, feeding tubes, Plexiglas cylinder (height-20 cm, diameter-15 cm), distilled Water, towels, warming lamp, horizontal ring-stand bar, adhesive tape, stopwatch, marker, measuring tape, petroleum ether, methanol, soxhlet apparatus, plant extracts, mixer grinder, evaporating dish, gum...
Acorus calamus, using soxhlet apparatus. The extracts obtained were defatted with petroleum ether. The plants were extracted separately with methanol after defatting with a mixture grinder. The powdered materials of the two plants were extracted separately with methanol after defatting with petroleum ether using soxhlet apparatus. The extracts obtained were evaporated, scraped out and stored in an airtight container. The yield obtained was 10% and 6% for Sapindus marginatus and Acorus calamus, respectively [8].

Phytochemical screening

The preliminary phytochemical tests of the plant extracts were carried out using standard procedures [9,10].

Experimental animals

Healthy albino mice of either sex weighing approximately 25-30 g were obtained from the Animal House, RMS, Imphal. These animals were acclimatized to the laboratory conditions for 7 days before the experiment. The animals were housed in the departmental animal room in groups in polypropylene cages at room temperature with natural light and dark cycle. They were housed in groups of six animals per cage and maintained on a standard animal diet with water ad libitum.

Acute toxicity testing

The acute toxicity testing was carried out as per OECD guidelines 423[11] in albino mice. Three animals were used for each step. The plant extracts i.e. MESE (methanol extract of Sapindus marginatus) and MEAC (methanol extract of Acorus calamus) were administered to the fasted mice at a dose of 300 mg/kg p. o. and observed once in every 30 min during the first 24 h and thereafter, daily for 14 days. As there was no mortality, the procedure was repeated with a higher dose of 2000 mg/kg, and the animals were observed for mortality and toxic symptoms. It was observed that the dose of 2000 mg/kg p. o. of the plant extracts caused no mortality or toxic symptoms in the tested animals and the dose was considered safe. Two doses of 200 mg/kg (1/10th of the maximum test dose) and 400 mg/kg (1/5th of the maximum test dose) of the plant extracts were selected as working doses for the experiment.

Selection of animals

Animals were subjected to a pre-test assessment. For the Forced swim test (FST), mice were placed gently and slowly by holding its tail in the water inside a vertical Plexiglas cylinder containing fresh water (25 ±1 °C) up to a height of 12 cm. The duration of immobility (sec) in various groups treated with Sertraline -10 mg/kg, 200 mg/kg and MEAC -200 mg/kg was significantly (P < 0.001) less when compared with the control. The period of immobility in the groups treated with 400 mg/kg of MESE and MEAC and Sertraline-10 mg/kg was comparable. However, the Sertraline treated group demonstrated significantly shorter (P < 0.001) period of immobility when compared with the groups treated with 200 mg/kg of MESE and MEAC. The extracts at the dose of 400 mg/kg demonstrated significantly (P < 0.001) shorter duration of immobility when compared with their lower doses i.e. 200 mg/kg. In the MESE-400 mg/kg treated group, the immobility period was significantly (P < 0.001) less when compared with the groups treated with MEAC-200 mg/kg, and the so also duration of immobility was shorter significantly (P<0.01) in the MEAC-400 mg/kg treated group. Therefore, the dose of 200 mg/kg was considered as an index of antidepressant activity. The animal was judged to be immobile when it remains floating passively in the water in a slightly hunched but upright position with its nose just above the surface. The swimming session, the mice were taken out of water, mopped with towels and kept warmed using lamps to prevent hypothermia before being returned to the cages. Water in the cylinder was changed after each swimming session.

Tail suspension test

The test was performed according to the method described by Duszczzyk M et al.[13] The animals were hung by the tail from a horizontal ring-stand bar 30 cm above the surface with an adhesive tape placed 2 cm away from the tip of the tail. The duration of immobility was observed for a period of 6 min. Mice were considered immobile only when they hung passively and completely motionless. The period of immobility in the control and various treated groups were compared. Decrease in the immobility period was considered as an index of antidepressant activity.

Analysis of results

The results were expressed as mean±standard deviation (SD) and analyzed by One-way analysis of variance (ANOVA) followed by Bonferroni test. P-value less than 0.05 was considered significant. IBM SPSS statistics version 21 was used for the analysis.

RESULTS

Phytochemical screening

The preliminary qualitative phytochemical analysis of methanol extract of Sapindus marginatus revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, gums and proteins, while the extract of Acorus calamus revealed the presence of flavonoids, saponins, tannins, alkaloids and starch.

Forced swim test

The mean duration of immobility (sec) in various groups treated with control, Sertraline-10 mg/kg, MESE-200 mg/kg, MESE-400 mg/kg, MEAC-200 mg/kg and MEAC-400 mg/kg were 110.06±13.38, 43.3±17.61, 110.06±13.38 and 54.3±17.61 sec respectively. In the groups treated with Sertraline-10 mg/kg and MEAC, the duration of the immobility decreased significantly (P<0.001) when compared with the control. The period of immobility in the groups treated with 400 mg/kg of MESE and MEAC and Sertraline-10 mg/kg were comparable. However, the Sertraline treated group demonstrated significantly shorter (P<0.001) period of immobility when compared with the groups treated with 200 mg/kg of MESE and MEAC. The extracts at the dose of 400 mg/kg demonstrated significantly (P<0.001) shorter duration of immobility when compared with their lower doses i.e. 200 mg/kg. In the MESE-400 mg/kg treated group, the immobility period was significantly (P<0.001) less when compared with the groups treated with MEAC-200 mg/kg, and the so also duration of immobility was shorter significantly (P<0.01) in the MEAC-400 mg/kg treated group. Therefore, the dose of 200 mg/kg was considered as an index of antidepressant activity.
mg/kg treated group when compared with MESE-200 mg/kg treated group. However, the immobility periods in the group treated with equal doses of the extracts were comparable (Table 1).

### Table 1: Effect on the duration of immobility in forced swim test (FST)

| Treatment group     | Duration of immobility (second) |
|---------------------|---------------------------------|
| Control-1% gum acacia susp. | 177.67±15.83                   |
| Sertraline-10 mg/kg  | 56.83±4.75                     |
| MESE-200 mg/kg      | 93.67±13.11                    |
| MESE-400 mg/kg      | 54.33±17.61                    |
| MEAC-200 mg/kg      | 110.00±6.38                    |
| MEAC-400 mg/kg      | 63.00±13.08                    |
| One way ANOVA       | F83.1                           |
|                     | Df35                            |
|                     | P<0.001                         |

Values are mean±SD, n=6; *P<0.01 when compared with control; †P<0.001 when compared with MESE-200 mg/kg and MEAC-200 mg/kg; ‡ P<0.01 when compared with 200 mg/kg of same extract. †P<0.01 when compared with MEAC-200 mg/kg; ‡ P<0.01 when compared with MESE-200 mg/kg (One way ANOVA followed by Bonferroni test).

### Tail suspension test

The mean duration of immobility (sec) in various groups treated with control, Sertraline-10 mg/kg, MESE-200 mg/kg, MESE-400 mg/kg, MEAC-200 mg/kg and MEAC-400 mg/kg were 166.33±4.50, 57.83±5.60, 144.83±28.28, 106.67±7.50, 153.50±8.46 and 114±4.94 respectively. The durations of immobility were significantly reduced in the Sertraline, MESE and MEAC treated groups when compared with the control group. The Sertraline treated group shortens the period of immobility significantly (P<0.001) when compared with the groups treated with 200 and 400 mg/kg of the two extracts. The group receiving 400 mg/kg of the extracts demonstrated a significantly shorter (P<0.001) duration of immobility when compared with their lower doses i.e. 200 mg/kg. In the MESE-400 mg/kg treated group, the immobility period was significantly (P<0.001) decreased when compared with the group treated with MEAC-200 mg/kg. Significantly short (P<0.01) immobility time was also observed in MEAC-400 mg/kg treated group when compared with the MESE-200 mg/kg treated group. The immobility periods in the group treated with equal doses of the extracts were comparable.

### Table 2: Effect on duration of immobility in tail suspension test

| Treatment group(p. o.) | Duration of immobility (second) |
|-------------------------|---------------------------------|
| Control-1% gum acacia susp. | 166.33±4.50                   |
| Sertraline-10 mg/kg  | 57.83±5.60                     |
| MESE-200 mg/kg      | 144.83±28.28                   |
| MESE-400 mg/kg      | 106.67±7.50                    |
| MEAC-200 mg/kg      | 153.50±8.46                    |
| MEAC-400 mg/kg      | 114±4.94                      |
| One way ANOVA       | F208.2                         |
|                     | Df35                            |
|                     | P<0.001                         |

Values are mean±SD, n=6; *P<0.01; †P<0.05 when compared with control; †P<0.01 when compared with MESE and MEAC treated groups; ‡ P<0.01 when compared with 200 mg/kg of the same extracts; ‡P<0.01 when compared with MEAC-200 mg/kg; ‡ P<0.01 when compared with MESE-200 mg/kg (One way ANOVA followed by Bonferroni test).

### DISCUSSION

Mice are commonly used rodents which are small, easily housed and maintained. Interestingly, the genetic, biological and behavioural characteristics of the rodents closely resemble to those of humans. Various human disorders, including behavioural disorders and seizures are studied in suitable mice models [13].

The Forced swim test (FST) and Tail suspension test (TST) are widely accepted behavioural models for the assessment of antidepressant activity. It is well known that many antidepressant drugs are able to reduce immobility time in rodents in the above models [14]. The forced swimming induced state of immobility in animals is claimed to represent a condition similar to human depression [15]. However, TST is considered to have greater pharmacological sensitivity than FST for antidepressants acting through selective inhibition of serotonin reuptake [16]. Both the methanol extracts of Sapindus marginatus and Acorus calamus at the two different doses of 200 and 400 mg/kg demonstrated decreased immobility time when compared with the control in both animal models and this observation is strongly suggestive of their antidepressant property. The extracts at the higher dose of 400 mg/kg is more effective than the 200 mg/kg dose. However, the antidepressant activity was not significantly different between similar doses of the plant extracts.

The biochemical basis of depression suggests that the disorder is linked with functional deficiency of the brain monoaminergic transmitters like norepinephrine (NE), 5-HT (serotonin) and/or dopamine (DA) [17]. Practically all antidepressants affect monoaminergic transmission in the brain in one way or the other and a large number of antidepressants with effects on reuptake/metabolism of biogenic amines, and on pre/post-junctionalaminergic/cholnergic receptors have also been known. The standard drug, sertraline is a popular antidepressant that blocks the reuptake of serotonin into the neurons resulting to increased serotonin availability at its receptors in the CNS [18].

The qualitative analysis of methanol extract of Sapindus marginatus and Acorus calamus revealed the presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins and carbohydrates. The flavonoids such as hesperidin, naringenin, quercetin, and astilbin are reported to display antidepressant-like activity in animal experimental models [19]. Many flavonoids are inhibitors of MAO-A and-B [20].

Therefore, it may be assumed that the presence of the flavonoids in the two plant extracts might have contributed to the antidepressant activity.

### CONCLUSION

The findings of our study reveal that the methanol extracts of Sapindus marginatus and Acorus calamus leaves possess antidepressant activity. Further studies with isolated biologically active principles of the plants in different models of depression will be more meaningful and interesting.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

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CONFLICTS OF INTERESTS
Declared none

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