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Antioxidant, analgesic, anti-inflammatory and CNS depressant activities of ethanolic and combined extracts of *Pandanus foetidus* and *Alangium salvifolium*: *in vitro* and *in vivo* studies

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Key words: Combined extract, analgesic, anti-inflammatory, CNS depressant

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Abstract

The present study investigated the phytochemical, antioxidant and in vivo biological activities such as anti-inflammatory, analgesic and CNS depressant activities of the ethanolic extracts of *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and combined extract of these plant species. In the DPPH radical scavenging activity assay, IC_{50} value of 28.5 µg/ml was found for the combined extract whereas the total antioxidant capacity (0.94±0.3) of that extract was concentration-dependent. All the extracts contained polyphenols and flavonoids but higher values of total phenolic (33.33 ± 5.06 mg/g) and total flavonoid (318.4 ± 4.39 mg/g) content were determined for the combined extract. The combined extract showed higher writhing inhibition (81%; 100mg/Kg body weight) among all the extracts which is quite comparable to the standard drug diclofenac sodium (87.17%; 10mg/Kg body weight). Anti-inflammatory activity of all the extracts was found to be moderate, but the combined extract showed higher activity (87.5% at 100 mg/kg body weight) in mice when compared to the standard (91.25% at 10 mg/kg body weight). The CNS depressant activity of combined extract was 39.75±2.63 at 90 min after administration of the extract, which was 28.25±1.46 for the standard. Our findings suggested that the combined extract of these species can be used as an herbal remedy in pain, inflammatory and CNS disorders. This is the first time that phytochemical and pharmacological activities of combination of extracts of these plant species have been reported.

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Introduction

Oxygen is the key element of our life which is used by the cells for energy production and thus free radicals are produced at a consequence in different physiological regions (Valko *et al.*, 2007, Halliwell & Gutteridge, 2007). At high concentrations, they produce oxidative stress that can damage all the vital cell structures through the chain reaction (Arulselvan *et al.*, 2016) and initiate a wide range of degenerative diseases like cardiovascular disease, inflammatory bowel diseases, asthma, arthritis, burn injury, cancer, β-thalassemia and sickle cell disease, Alzheimer's disease, degenerative eye disease etc. (Valko *et al.*, 2007, Biswas *et al.*, 2017, Harrison *et al.*, 2003, Rahman *et al.*, 2012, Ríos-Arrabal *et al.*, 2013).This oxidative stress is linked with inflammation and responsible for a number of chronic diseases like depression (Pasco *et al.*, 2010, Leonard *et al.*, 2012, Ambade *et al.*, 2012).

Oxidative stress can be prevented or minimized by the action of antioxidants that can inhibit the chain reaction of free radicals as well as are capable to avert their harmful physiological effects (Sun *et al.*, 2002). Medicinal plants are excellent sources of natural antioxidants possessing a wide range of pharmacological properties (Kin *et al.*, 2018). Poloyphenol and flavonoids act as natural antioxidants in evidence-based manner with a range of medicinal properties like anti-allergic, anti-inflammatory, analgesics, vasodilating, antimicrobial and anticancer activities (García-Pérez *et al.*, 2019, Ghasemzadeh & Jaafar, 2013). Different classes of synthetic drugs are available for the treatment of physiological disorders but they are not equally effective in every case because of diverse adverse effects like gastrointestinal discomfort, liver and kidney damage, cardiovascular effect, mutagenesis and other complicacies (Paul *et al.*, 1999). Therefore, phytochemists all over the world are looking for new drugs from plant origins as alternatives to synthetic analgesic and anti-inflammatory agents to minimize the side effects (Nisar *et al.*, 2017). Even natural antiviral compounds have been proven to be more effective against COVID-19 as compared to synthetic drugs with fewer side effects (Farooq & Ngaini, 2021). At least 25% of prescribed drugs contain herbal medicines (Rates *et al.*, 2001). For primary health care, herbal medicines are very much demandable worldwide due to wide biological and medicinal values, higher safety edges, and lesser expenses (Cragg *et al.*, 1997, Duthie *et al.*, 2000). But the action of all antioxidants is not the same because free radicals act differently in different physiological regions. This is the reason why a single antioxidant is not sufficient to combat every free radical. According to F. Zhu *et al.* the potency of combined active ingredients from natural products is quite equal comparing to synthetic drugs (Zhu *et al.*, 2012). Now a days the traditional medicinal system established its efficiency through the use of combined herbal therapy (Otieno *et al.*, 2008, De *et al.*, 2017).It became evident that combinations of plant extracts from different spices potentiate or enhance the effect of each other to produce therapeutic synergism (Williamson *et al.*, 2001).

Pandanus foetidus (local name: Keya) belongs to the family pandanaceae. According to literature, the plant exhibited potent antibacterial activity against some selected bacteria (Uddin *et al.*, 2008). Leaf part of this medicinal plant exhibited antinociceptive, anti-inflammatory, analgesic, antidiarrheal, cytotoxic, CNS depressant and thrombolytic activities to heal a number of diseases (Lokman *et al.*, 2013, Islam *et al.*, 2013, Rahman *et al.*, 2015, Hossen *et al.*, 2014). The plant *P. foetidus* possesses significant bioactive principles (Sikder *et al.*, 2013) and phytochemical screening of the plant indicated to the presence of carbohydrates, saponins, tannins, glycosides, steroids, alkaloids, polyphenol and flavonoids that are responsible for the antioxidant activity of this plant species (Lokman *et al.*, 2013, Nurunnahar *et al.*, 2017).

Alangium salviifolium or 'Akorkanta', belonging to the cornaceae family, is a flowering plant. It is a well-known traditional medicine having anti-tubercular, antispasmodic and anti-cholinesterase effects (Warrier *et al.*, 1993). In rheumatism, haemorrhoid

and treatment of bites by snake, rabbits, rats and dog the roots and fruits are very effective (Narayana *et al*., 2003, Tanwer & Vijayvergia, 2014). Among various bioactive compounds, 'ankorine', a new alkaloid, has been isolated from leaves of this plant (Jain *et al*., 2002). Like others parts, the flower also possesses anticancer, antinociceptive, anti-inflammatory and CNS depressant activities (Zahan *et al*., 2011, Zahan *et al*., 2013, Zahan *et al*., 2012). According to the literature, flower contains antibacterial compounds responsible for its antibacterial activity (Anjum *et al*., 2002).

In this work, we provided efforts towards the best use of medicinal plants by combining the leaf and flower extracts from *Pandanus foetidus* and *Alangium salvifolium*, respectively. Antioxidants, phytochemical and pharmacological properties of this combined extract were investigated for the first time. To the best of our knowledge, evaluation of medicinal properties of the combination of above-mentioned plant extracts has not been reported yet.

Materials and methods

Preparation of crude extracts

After collection of fresh leaves and flowers of *Pandanus foetidus* and *Alangium salvifolium*, four different mixtures were prepared from: (a) leaves of *Pandanus foetidus* (b) leaves of *Alangium salvifolium* (c) flowers of *Alangium salvifolium* and (d) leaves of *Pandanus foetidus*, leaves and flowers of *Alangium salvifolium* (combined mixture).

The mixtures were grinded into powder and compounds were then extracted from each mixture with ethanol using the cold extraction process (Abah & Egwari, 2011). After filtration, the ethanol extracts were concentrated with a rotary evaporator.

In Vitro Antioxidant Assays

DPPH radical scavenging assay

Free radical scavenging activities of the extracts were determined by measuring the changes in absorbance of DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) with a spectrophotometer (Choi *et al*., 2000).

Determination of total phenolic content

Total phenolic content of all four extracts of *Pandanus foetidus* and *Alangium salvifolium* was determined with Folin–Ciocalteu Reagent (FCR), to measure their reducing capacity (Singleton & Rossi, 1965).

Determination of total flavonoids

Total flavonoid content of the ethanol extracts was determined by aluminum chloride colorimetric method in which aluminum chloride forms complex with hydroxyl groups of flavonoids present in the samples. The maximum absorbance value was measured at 510 nm (Dewanto *et al*., 2002).

Total antioxidant capacity

The total antioxidant activity of the extracts was evaluated by the phosphomolybdate method (Prieto *et al*., 1999).

In vivo pharmacological activities

Experimental animals and their grouping

Swiss albino male mice (3-4 weeks old, weighing 20-27 grams) were bought from the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. They were kept in standard environmental conditions before initiating the experiment. Randomly selected animals were divided into six groups as follows: (a) control (b) standard(c) receiving extract of leaves of *Pandanus foetidus* (d) receiving extract of leaves of *Alangium salvifolium* (e) receiving extract of flowers of *Alangium salvifolium* and (f) receiving the combined extract of leaves of *Pandanus foetidus*, leaves and flowers of *Alangium salvifolium*. There were four mice in each group and each mouse was weighed properly and the doses of the test samples and standard materials were adjusted according to their body weight.

Ethics approval and consent to participate

Ethical clearance of the experiments using Swiss albino mice was provided by the Institutional Animal, Medical Ethics, Bio-safety and Bio-security Committee (IAMEBBC) for Experimentations on Animals, Human, Microbes and Living Natural

Sources (Memo No. 415(27)/320/IAMEBBC/IBSc), Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh.

Study of analgesic activity

Acetic acid-induced writhing test

Acetic acid-induced writhing method was used to evaluate the analgesic activity in which each group received 0.7% v/v acetic acid 30 min after oral administration of extracts and injection of standard drug (diclofenac Na) (Sharma *et al.*, 2010). After a given time interval, the mice were observed for specific body contraction which is known as writhing.

The number of writhing during the following 20 min period was counted. The percentage of inhibition (% analgesic activity) was calculated following the equation below:

$$\% \text{ inhibition} = (N-N^t)/N \times 100$$

Here, N = Average number of stretching of control per group

N^t = Average number of stretching of test per group

Study of Anti-inflammatory activity

Carrageenan-induced paw edema test

Anti-inflammatory activity of test samples was determined by carrageenan induced paw edema method in which carrageenan was used to produce inflammation (Elisabetsky *et al.*, 1995).

The test samples, standard and carrageenan were injected to the left hind paw of every animal. The degree of edema induced was assessed. The right hind paw served as a reference to make correlation.

Study of CNS depressant activity

Hole cross method

The CNS depressant activity of the plant extract was carried out by the well-known hole cross method which characterizes the emotional behavior of experimental animals by counting the number of passages of mice through the hole of a wooden cage after administration of test sample and standard drug diazepam (Paul *et al.*, 2022).

Open field method

The open field test is most common and frequent method to conduct CNS depressant activity that was carried out in this study by using a wooden board of open field with a series of alternatively colored black and white. The number of squares accessed by the animals was counted after administration of tests and standard drug diazepam (Paul *et al.*, 2022).

Statistical analysis

The data were expressed as the mean ± SEM of three replicate experiments. The analysis was done using Statistical Package for the Social Sciences (SPSS) in the version of 15.0; SPSS Inc, Chicago. All the values were subjected to ANOVA followed by Dunnett's test and $p<0.01$ were considered to be statistically significant. Data shown are mean ± SE (n=4). Statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) differences from the control are indicated with asterisks.

Results

Determination of the Antioxidant activity Assays

DPPH radical scavenging assay

DPPH was used to evaluate the free radical scavenging capacity of *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and combined extracts. At a concentration of 100µg/ml, the free radical scavenging activity of *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and combined extracts was 78.95±1.53%, 73.24±6.10%, 83.95±4.15 and 88.24±3.86%. Whereas at the same concentration, the standard BHT (Butylated Hydroxy Toluene) was 95.21 ± 9.19%. The results of DPPH radical scavenging assays of different extracts and standard presented in following figures (Fig.1 and Fig. 2) conclude that, the combined extract showed higher radical scavenging activity (28.5µg/ml) than other extracts.

Determination of total phenolic content

Phenolic content of the samples was estimated on the basis of the standard curve for gallic acid as shown in Fig. 3. Combined extract possesses the higher phenolic content (33.33mg of GAE/gm of dried

extract) among the extracts. These phenolic components possibly contributed directly to its antioxidant activity.

Determination of total flavonoids

Total flavonoid content of the samples was calculated on the basis of the standard curve for catechin as shown in Fig. 4. Higher amount of flavonoids (318.4± 4.39 mg/g of catechin) were present in the combined extract comparing to extracts from other samples.

Determination of total antioxidant capacity

The total antioxidant capacity of the standard (ascorbic acid) and plant extracts were shown in Fig. 5. The combined extract exhibited maximum antioxidant activity in a concentration-dependent manner.

Determination of analgesic activity

Acetic acid-induced writhing test

In acetic acid induced writhing test, all extracts showed significant activity and dose dependently suppressed the frequency of acetic acid-induced writhing in mice (Table 1). From the table, it becomes evident that the combined extract showed significant writhing inhibition comparing to the standard drug diclofenac Na. *Alangium salvifolium* (Leaf extract) also showed similar degree of activity.

Table 1. Analgesic activity of extracts from *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and their combinations in the writhing method.

Sample	Dose (mg/kg)	Writhing Number	Inhibition of Writhing (%)
Control	-	39.0±1.0	0
Diclofenac Na	10	5.0±1.0***	87.17%
Pandanus foetidus (Leaf extract)			
Group –I	50	26.5±.02	32%
Group-II	100	24.18±.01**	38%
Alangium salvifolium (Leaf extract)			
Group –I	50	12.09±1.01**	69%
Group-II	100	7.8±1.01*	80%
Alangium salvifolium (Flower extract)			
Group –I	50	17.16±.03	56%
Group-II	100	8.6±00**	78%
Combined extract			
Group –I	50	11±1.02**	72%
Group-II	100	7.41±1.01**	81%

Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

Study of anti-inflammatory activity

Carrageenan induced paw edema method for anti-inflammatory activity

Carrageenan induced paw edema method is a well-known procedure for the determination of anti-inflammatory activity. From the result it is shown that the combined extract exhibited higher anti-inflammatory activity (87.5%) at a dose of 100mg/kg body weight which is quite comparable with standard drug indomethacin (91.25%) after 3hr of administration of test samples (Fig. 6).

Determination of the Central nervous system (CNS) depressant activity

Open field test

In open field test, the extracts showed a noticeable decrease in locomotion in the test animals and an exciting result was found (Fig. 7). From the result, it was observed that number of movements decreased in test animals receiving the combined extract. The value (39.75±2.63) was higher than other extracts (the minimum value was 50.25±3.4 for *Alangium*

salvifolium (flowers) and lower than the value obtained for standard drug diazepam (28.25±1.46).

Hole cross test

The test animal treated with all extracts under study showed dose dependent reduction in the locomotor activity. Results for the combined extract and *Alangium salvifolium* flower extracts were comparable with that of standard drug diazepam. The result evidenced that the test animals were showing significant decrease in number of movements at a dose of 100mg/kg body weight comparing to the standard at 90 minutes after administration of the extracts (Fig. 8).

Discussion

Phytochemical screening of both plant extracts, *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers), revealed the presence of several secondary metabolites like alkaloids, saponins, tannins, among other phenolic compounds which are responsible for the chemical and pharmacological activities of theses plant extracts (Lokman *et al.*, 2013, Tanwer & Vijayvergia, 2014).

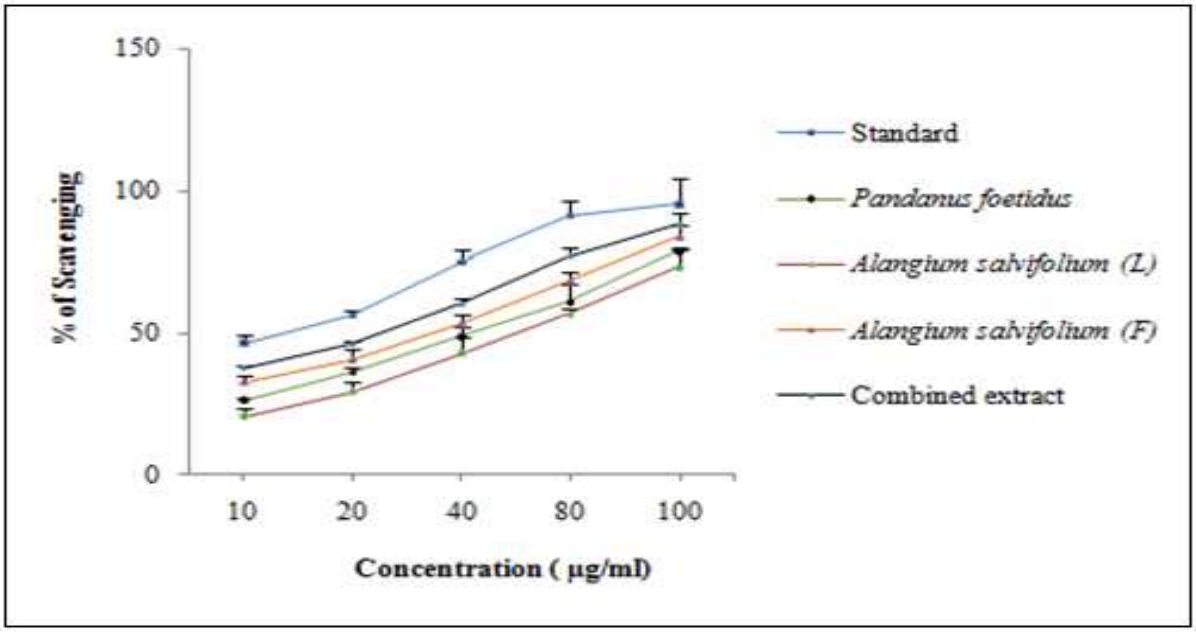

Fig. 1. DPPH radical scavenging activity of extracts from *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and their combination. BHT (Butylated Hydroxy Toluene) was used as a standard. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

The DPPH radical scavenging assay is frequently used to assess the antioxidant and free radical scavenging capacities of diverse test samples (Salazar *et al.*, 2008). In the free radical scavenging ability test, the IC_{50} value of BHT (standard) and samples [*Pandanus foetidus* (leave), *Alangium salvifolium* (leave and flower) and their combined extract] were 12.5µg/ml, 39µg/ml, 58µg/ml, 41µg/ml and 28.5µg/ml, respectively. After comparing with the standard, it became clear that plant extracts possess very mild antiradical activity. With an IC_{50} value of 28.5µg/ml, the combination extract outperformed the other extracts in terms of radical scavenging ability. According to earlier research, the methanol extract of *Pandanus foetidus* has IC_{50} values of 115.60 µg/ml that showed moderately effective against DPPH free radical scavenging action, while the IC_{50} value of petroleum ether extract, ethyl acetate extract and methanolic extract of *Alangium salvifolium* was found to be 94.78 µg/ml, 85.78 µg/ml and 49.23 µg/ml, respectively that is similar to the results of our experiment (Nurunnahar *et al.*, 2017, Bhosale & Singh, 2020). Total phenolic content of the extracts was determined from the standard curve of gallic

acid. Combined extract possesses the higher phenolic content among the extracts. The phenolic compounds found in plants have strong antioxidant capabilities (Dehshahri *et al.*, 2012). At 100µg/ml concentration, the phenolic content was found to be 33.33 ± 5.06 mg/g in combined extract whereas 45.75±5.69 mg/g and 152.73 ± 13.60 mg/g of gallic acid were found in *Pandanus foetidus* and *Alangium salvifolium*, respectively (Nurunnahar *et al.*, 2017, Zahan *et al.*, 2013) which contributed to the antioxidant property of these plant extracts.

According to another finding, the total phenolic content of ethanol and aqueous extract of *Alangium salvifolium* were 74.21 mg/g and 56.48 mg/g of GAE, respectively (Patel & Manigauha, 2018). Much higher value (269 mg/g of GAE) has also been reported for *Pandanus foetidus* (Aovi & Rahman, 2019).

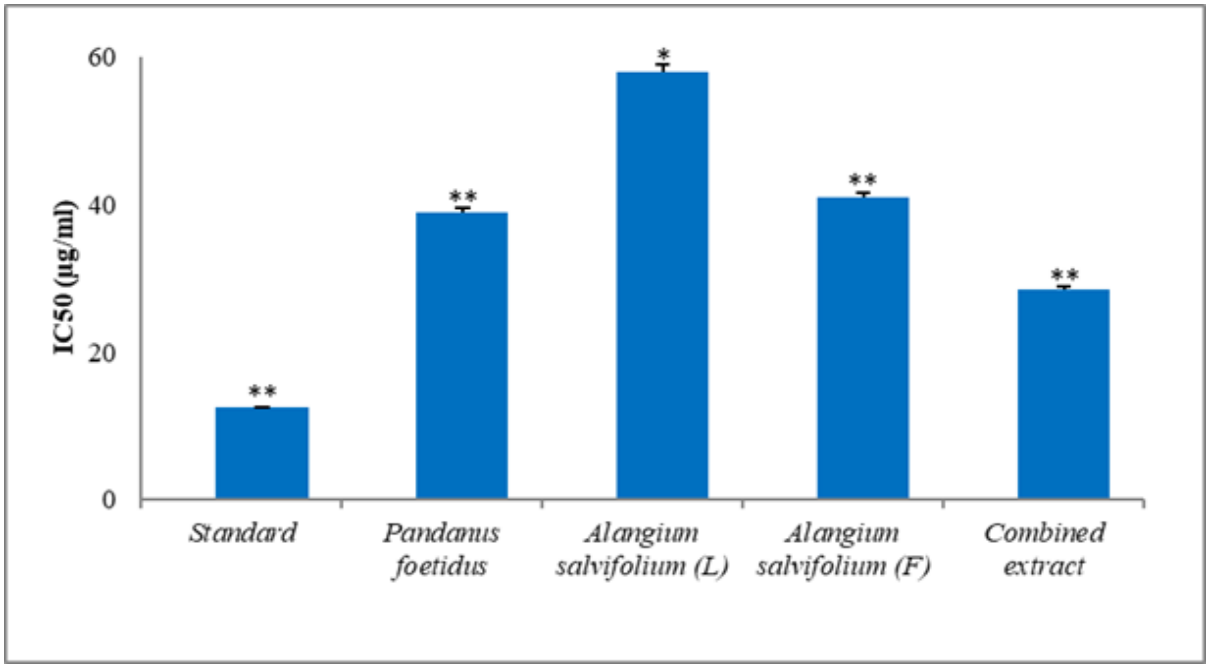

Fig. 2. IC_{50} (µg/ml) values of standard (BHT) and extracts from *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and their combination for the hydroxyl radical scavenging activity. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

The flavonoid content of the extracts was determined from a standard curve of catechin. The only metabolites that exhibit pharmacological action are frequently flavonoids (Arct & Pytkowska, 2008). Numerous antibacterial, antiviral, anti-inflammatory, anti-cancer, and anti-allergic effects have been observed in studies on flavonoid derivatives (Montoro *et al.*, 2005). A previous study suggested that the methanol extracts of *Pandanus foetidus* has flavonoid content of 295.27 ±6.29 mg/g of catechin, whereas the concentration of flavonoids found in *Alangium salvifolium* were 59.26 mg/gm of catechin (Nurunnahar *et al.*, 2017, Patel & Manigauha, 2018). Though the earlier research results differ from our findings, all these studies confirm the presence of antioxidant property in both these plants because of their high phenolic content *(*Aovi & Rahman, 2019*,* Shravya *et al.*, 2017*)* and the presence of phenols and flavonoids was linked to the bioactivity of several plants from the genera of *Pandanus* and *Alangium salvifolium* (Nurunnahar *et al.*, 2017, Shravya *et al.*, 2017, Jain & Jain, 2011). The acetic acid-induced writhing test is highly recommended due to its sensitivity and ability to detect antinociceptive effects of natural products, in which the abdominal constriction was associated with the identification of peripheral analgesic activity of the extracts (Subedi *et al.*, 2016).

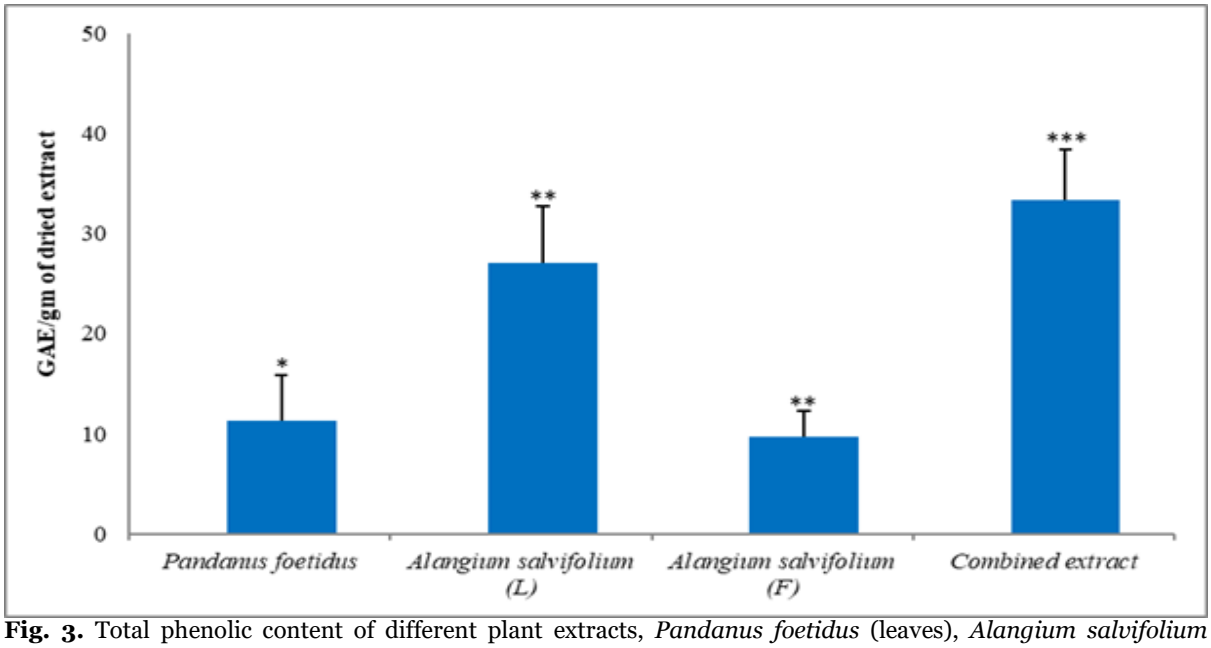

Fig. 3. Total phenolic content of different plant extracts, *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and the combined extract. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard (Gallic acid).

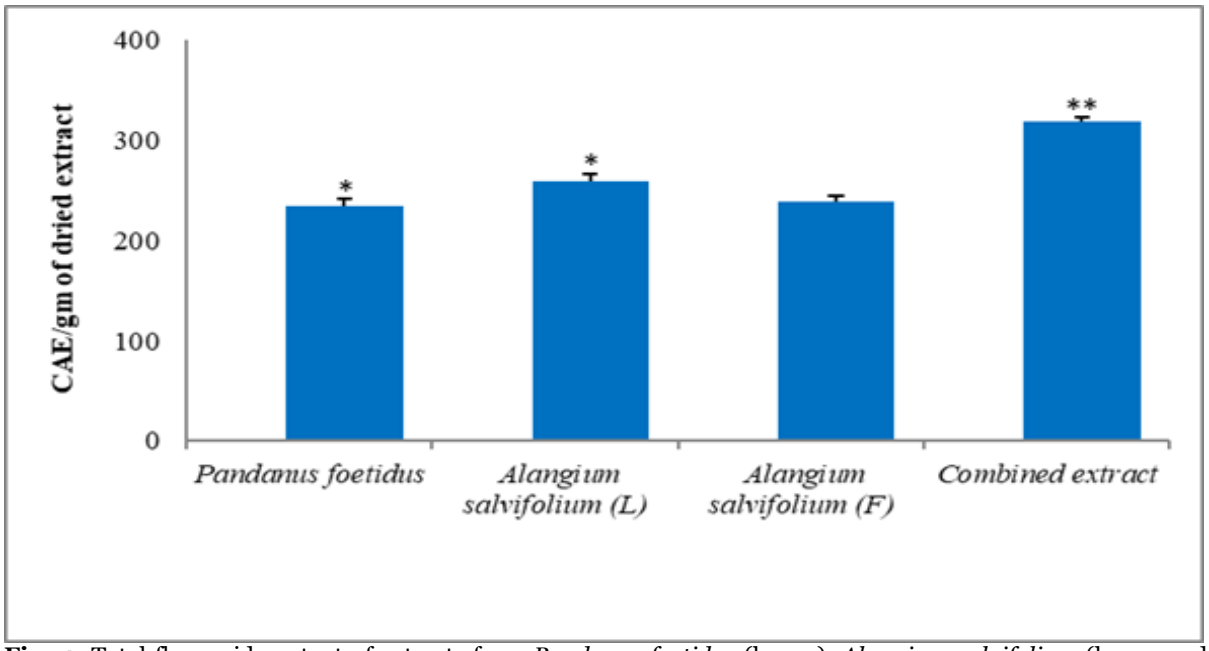

Fig. 4. Total flavonoid content of extracts from *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and their combination. Catechin was used as the standard here. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

The peritoneal cavity is irritated and stimulated by intraperitoneal injection of acetic acid, which results in the production and release of a number of endogenous inflammatory mediators, including histamine, serotonin, bradykinin substance P, and PGs, which induced visceral pain (Konaté *et al.*, 2012). Additionally, this model Increases the levels of PGE and PGF2a, that enhance capillary permeability and thus activates the afferent nociceptors, which have also been linked to aggravate inflammatory pain (Demsie *et al.*, 2019). In our experiment, at 100 mg/kg body weight, *Pandanus foetidus* (leave), *Alangium salvifolium* (leave and flower) and combined extract showed 38%, 80%, 78% and 81%

writhing inhibition, respectively while at 10 mg/kg body weight, the standard drug diclofenac sodium showed 87.17% writhing inhibition. Therefore, the significant analgesic action of combined extract may be due to the presence of analgesic principle that interferes with prostaglandin pathway. Hence, the possible causes of the analgesic effect are associated with its anti-inflammatory action.

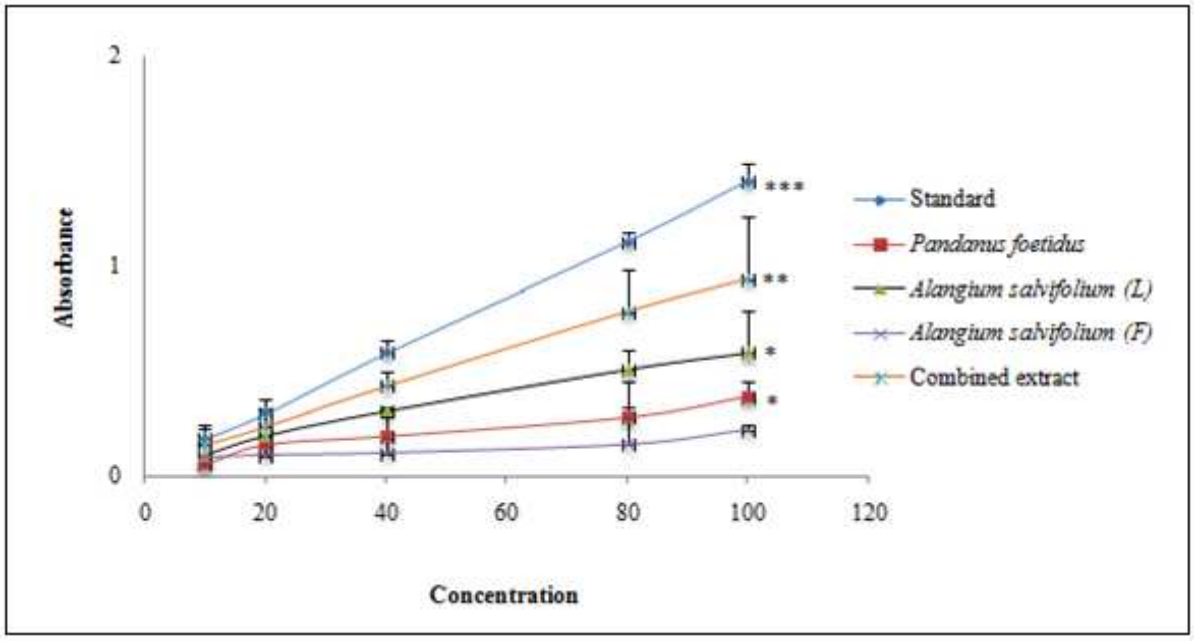

Fig. 5. Total antioxidant activity of *Pandanus foetidus* (leaves), *Alangium salvifolium* (leaves and flowers) and their combined extracts. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard (Ascorbic acid).

The inhibition of the production and release of numerous endogenous inflammatory mediators as well as the lowering of the sensitivity of peripheral nociceptors responsible for inducing pain may be the likely mechanisms by which the combined extract generated peripheral analgesia. Previous study finds that the methanol extracts of *Pandanus foetidus* produced 69.93% writhing inhibition at a dose of 500 mg/kg body weight (Uddin *et al.*, 2006) whereas the chloroform extract of *Alangium salvifolium* exhibited the writhing inhibition of 51.60±1.86 at 100 mg/kg body weight (Zahan *et al.*, 2013). Both experiments support the proposed mechanism that confirm the analgesic action of these plant extracts by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process.The biphasic carrageenan-induced paw edema test is frequently used to determine acute inflammation. The release of histamine and bradykinin during the early stages of inflammation encourages the growth of paw edema by expanding blood vessels (Sengar *et al.*, 2015). A crucial component of sustaining inflammation in the later stages of inflammation is the generation of prostaglandins, which is mediated by leukotriene and bradykinin (Marzouk *et al.*, 2010).

In this investigation, extracts treatments were dramatically decreased the volume of paw edema in mice, indicating that the extracts could prevent the development of the aforementioned inflammatory mediators. We already mentioned above that the investigated plants are rich in different phytochemicals like alkaloids, flavonoids, saponin, tannins, phenolic compounds, glycosides and so on.

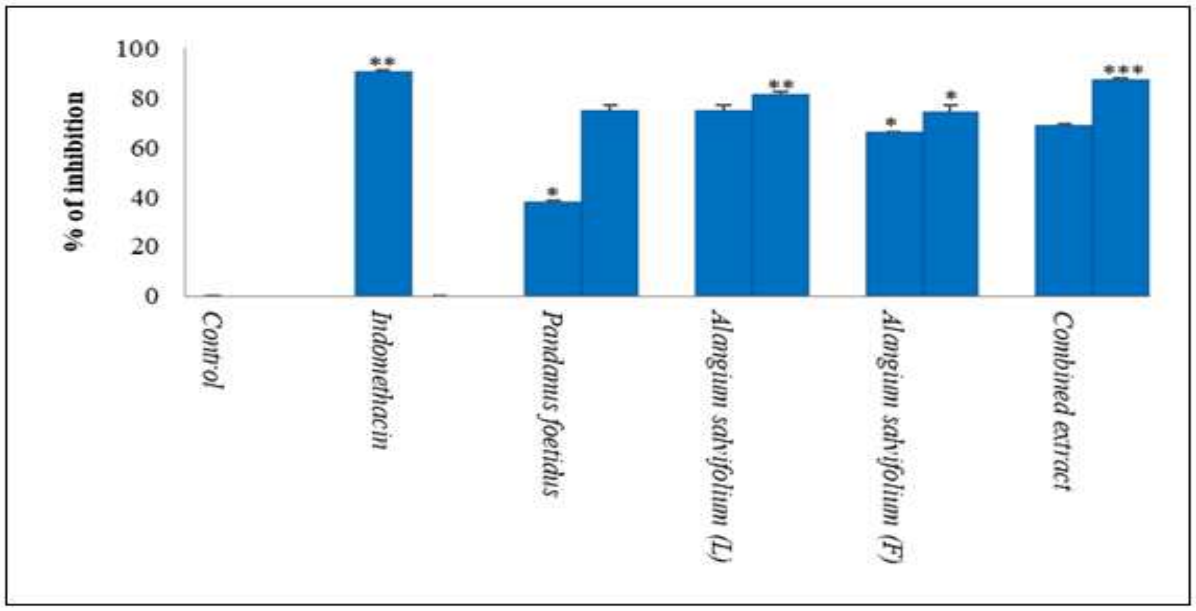

Fig. 6. Anti-inflammatory activity of *Pandanus foetidus* (L), *Alangium salvifolium* (L), *Alangium salvifolium* (F) and their combined extracts at 50 mg/kg and 100mg/kg body weight. Indomethacin was used as the standard drug. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

This is probably the reason why *Pandanus foetidus* and Alangium salvifolium showed significant anti-inflammatory effects of 53.33% and 49.55% respectively, (Nurunnahar *et al.*, 2023, Prajapati *et al.*, 2019) either by interfering with COX expression and production of PGE_2 or by inhibiting the activity and production of several inflammatory mediators (Demsie *et al.*, 2019, Geremew *et al.*, 2015).

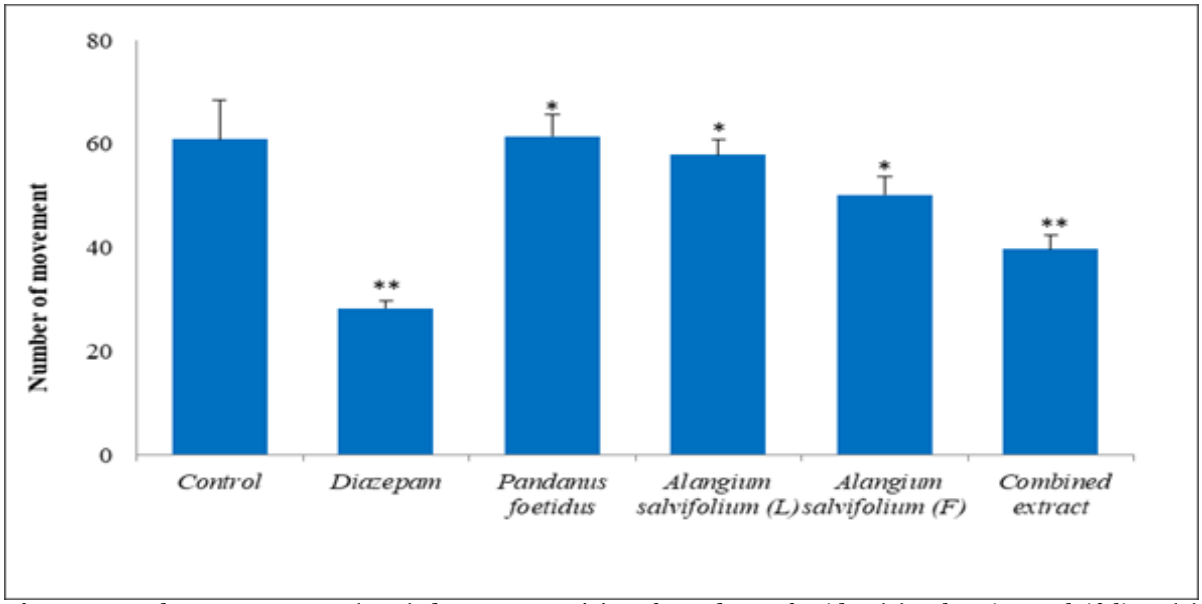

Fig. 7. Central nervous system (CNS) depressant activity of *Pandanus foetidus* (L), *Alangium salvifolium* (L), *Alangium salvifolium* (F) and their combined extract determined by the open field test at 100mg/kg body weight of mice. Standard drug Diazepam was used here. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

The combined extract possessed significant anti-inflammatory activity among all the extracts. Therefore, it can be assumed that the significant anti-inflammatory activity of the combined extracts may be caused by the cumulative effects of various active phytoconstituents in reducing the synthesis, release, and action of several endogenous inflammatory mediators that are crucial for the initiation and progression of inflammation. The CNS depressant effect of *Pandanus foetidus* (leave), *Alangium salvifolium* (leave and flower) and combined extracts were investigated using well-known neuropharmacological models namely open field and hole cross tests that considerably reduced the locomotion in test animal. The findings of our studies demonstrated that the extracts considerably decreased locomotor activity, confirming their CNS depressive effects. Locomotor activity is a marker of the CNS's excitability, and a decrease in it indicates CNS depressive activity (Dey *et al.*, 2011). Gamma-Amino Butyric Acid (GABA) is the primary inhibitory neurotransmitter in the CNS, via which a range of anxiolytic, muscle relaxant, and sedative-hypnotic exert their action.

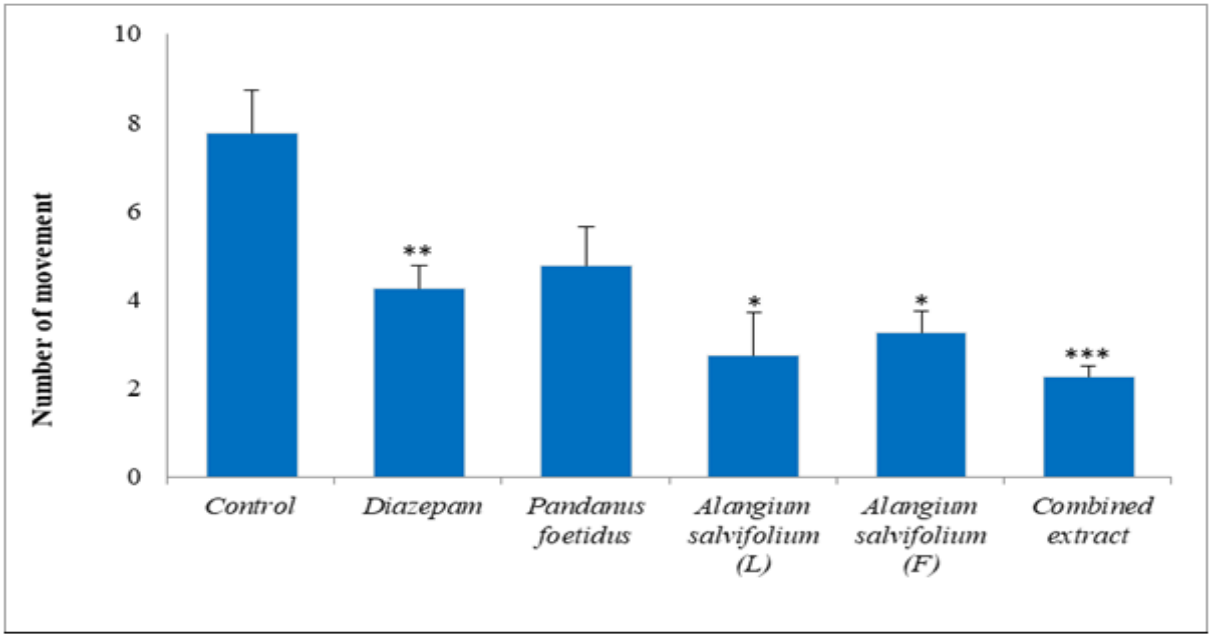

Fig. 8. Central nervous system (CNS) depressant activity of *Pandanus foetidus* (L), *Alangium salvifolium* (L), *Alangium salvifolium* (F) and their combined extract by the Hole Cross test at 100mg/kg body weight of mice. Diazepam was used as the standard drug. Data presented as Mean ± SD, P*<0.05, P**<0.01, P***<0.001 are statistically significant when compared to the standard.

The observed effects from *Alangium salvifolium* resemble with the drugs that act on GABA/ benzodiazepine receptor complex as well with drugs that stimulate glucocorticoid production and release in the adrenal cortex (Nishikawa *et al.*, 2004). In previous findings, plant extracts of *Pandanus foetidus* showed significant decrease in the number of movements of 11.5±4.20 in open field method and 2.0±2.82 in hole cross method (Geremew *et al.*, 2015). Similar experiments in open field (9 ± 0.58) and hole cross test result (0.67± 0.67) showed that the plant extract of *Alangium salvifolium* possesses CNS depressant activity (Tanwer & Vijayvergia, 2014). *A. salvifolium* extract may act by membrane hyperpolarization which potentiates GABAergic inhibition in the CNS (Khatun *et al.*, 2011). Prior phytochemical analysis proposed that, *P. foetidus* could have a CNS depressive impact because a wide variety of secondary metabolites were found in *P. foetidus* that is consistent with the proposed concept (Prajapati *et al.*, 2019). Furthermore, both plants are rich in flavonoids, which serve as ligands for GABA receptors in the central nervous system, causing a

CNS depressive effect similar to the benzodiazepine employed in the experiment (Dal Molin *et al*., 2012).

According to the results, all plant extracts, particularly when combined, significantly reduced the movement of test animals with increasing dose levels (50 mg/kg and 100 mg/kg body weight) in contrast to the control group, which is shown in Fig.7 and Fig.8, respectively. According to the research, the diverse secondary metabolites of medicinal plants are combined together through which they exert synergistic impact on distinct physiological site to produce corresponding actions (Briskin *et al*., 2000). This statement is aligned with our result that, the activity found from the combined extract at higher dosage levels, might be attributed to the presence of numerous phytochemicals in these plant species that provide synergistic effects in combined state.

Conclusion

Results from the present study suggested that, the combined extract of *Pandanus foetidus* (leave), *Alangium salvifolium* (leave) and *Alangium salvifolium* (flower) produced appreciable antioxidant, analgesic, anti-inflammatory and CNS depressant activity in a dose dependent manner which might be due to the bioactive compounds present in these plant extracts and all the results were quite comparable with the standard used in the experiments. Accordingly, it appears to be a potential combination of plant extracts for further investigation to understand its precise mode of action and isolation of active components responsible for the activity. Therefore, further study is needed to be conducted for evaluation of the possible mechanism of the combined plant extract which can open a new door of medicines.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

IH wrote the main study protocol and designed the study. N did all the experiments. N, BI and SK performed data analysis. AC, AM and IH supervised the data collection. N wrote the initial draft of the manuscript, which was revised by IH. BI, AC and AM provided administrative and logistics supports. All authors read and approved the final manuscript.

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