Evaluations of Antioxidant and Anxiogenic Like Activities of *Amomum aromaticum* Roxb. Leaves

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Abstract. The present study was planned to determine phytochemicals, antioxidants, and anxiogenic (anti-psychotic) activity of methanol extract of *Amomum aromaticum* roxb. Leaves (MEAA) in the laboratory using both *in vitro* and *in vivo* methods. *In vitro* antioxidant investigation was implemented by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, FeCl3 rfeducing power and total phenolic content study followed by established protocol described in previous. *In vivo* anxiogenic (anti-psychotic) activity was evaluated by Elevated Pluz Mazes (EPM) and Hole Board Test (HBT). The results showed the total phenolic content (36.56 ± 1.02 mg gallic acid/gm). In the anxiogenic activity, MEAA decreased the number of head dips in the hole-board test. In the case of the EPM test, this crude extract induced an anti-psychotic like effect rather than the anxiolytic effect in mice. To conclude these results suggested that this plant is a moderate source of antioxidants and it is not toxic. The results also demonstrate that MEAA possesses potent anxiogenic activity which could be used therapeutically for sleep disorders like hypersomnia as well as anti-psychotic disorders.

Keywords: *Amomum aromaticum*, DPPH (1, 1-Diphenyl-2-picrylhydrazyl), Free radical reducing power, Total phenolic content, Anxiety

Introduction

Antioxidants are compounds which have an oxidative chain reaction that breaks down properties. It has recently been an increasing interest in the potential therapeutic plants and herbs as antioxidants in re-antioxidants in minimizing tissue illness caused by oxidative stress. Ascorbic acid, carotenoids and phenolic compounds are more selective among the many natu rally occurring antioxidants (Veeru, Kishor, & Meenakshi, 2009). There is through understanding of the role of active oxygen and free radicals in tissue damage in various human diseases. Effective hydrogen, which is a radical hydrogen peroxide (H2O2), is a bi product of normal metabolism and assaults biomolecules, resulting in damage to cells and tissue Superoxide (V-) (Yen & Chen, 1995). Again anxieties are shown by disruptions in mood as well as of thought, actions and neurological activity which are understandable as the pathological equivalents of natural panic. Although not completely understood, the etiology of most anxiety disorders has recently become more apparent (Sonavane, Sarveiya, Kasture, & Kasture, 2002). About 450 million people lead to mental or psychological problems, according to the World Health Report (Organization, 2001) but only a small percentage is considered the most critical. This is 12.3% of the worldwide disease risk and will increase to 15% by 2020 (Reynolds, 2003). With regard not only to cultural traditions and biodiversity but also to community healthcare and clinical research, it is not only valuable for maintaining the cultural identity and biological diversity of their use for indigenous cultures. Treatments with synthetic regional formulations have several adverse effects and are not available to humans due to higher drug costs. Plants around us without scientific testing are used to overcome this problem. Medicinal plant work has advanced gradually in the search for new medicinal drug for neurological disorders around the world, showing pharmacology (Herrera-Ruiz et al., 2006). The pharmacology has been shown in the worldwide search for new medicinal drugs for the

management of oxidative and neurologic disorders. *Amomum aromaticum* roxb. (Family: Zingiberaceae) is also referred to as Bengal (Bengali) cardamom or padagro (marma tribe) used traditionally to provide salad dressing and foods for flavoring (Devi, Singh, & Das, 2014) and in Burma, India: Assame, Odisha; America, Asia, this plant has been also used as Scorpion antidote, and snakebite (Tushar, Basak, Sarma, & Rangan, 2010). This study examines the phytochemicals, anti-oxidants, and anxiety activity of the methanol extract of *Amomum aromaticum* roxb. leaves (MEAA).

Materials and Methods

Collection and proper identification

The leaves have been accumulated from Shitapahar, Kaptai, Chittagong and have been detected by Dr. Shaikh Bokhtear Uddin, Professor, Botany Department, Chittagong University, Bangladesh.

Drugs and chemicals

Diazepam was obtained from Square Pharmaceutical Ltd., Bangladesh, Tween 80, DPPH, gallic acid, phenol and methanol were attained from Sigma Chemicals Co., U.S.A.

Plant Material Extraction

Standard protocol established by Zhang J, et al. (Zhang et al., 2013) was implemented to prepare the extract.

Preliminary Phytochemical Analysis

Hot water extraction

Established protocol described by Evan, et al. (Evans, 2009) with slight modifications the hot water extraction was implemented.

Preliminary phytochemical screening procedure

The qualitative screening was performed in accordance with the established study protocol (Chakraborty, Devi, Rita, Sharatchandra, & Singh, 2004; Thamaraiselvi & Jayanthi, 2012) for glucose, cholesterol, protein, alkaloids, flavonoids, terpenes, cardiac glycosides, sterols, tannins, saponins, phlobatannins phenols, quinones, oxalates, fatty acids, anthocyanins, leucoanthocyanin products, coumarins, emodines, di-terpenes test.

In vitro antioxidant assay

DPPH free radical scavenging assay

Free radical scavenging of test samples was measured with the Spectro-photometric method described by Braca and al. (Montoro, Braca, Pizza, & De Tommasi, 2005) by measuring the radical change in absorption of DPPH (1, 1-diphenyl 2-picryl-hydrazil) of 517 nm.

Reducing power capacity

MEAA's reduction power was assessed using the established method described in (Aiyegoro & Okoh, 2010).

Determination of total phenolic compound

Total phenolic content of the MEAA was determined using the folin-Ciocalteau reagent method (Vasco, Ruales, & Kamal-Eldin, 2008) was actuated.

In vivo anxiogenic activity

Experimental animals

Swiss albino mice, weighing approximately 25–30 g, were collected from a venom research center in Chittagong, Bangladesh. The standard protocol (Bruce & Parkes, 1949) was used to support and prepare the animals for experiments. This study protocol has been approved by the "P & D Committee" of the Department of Pharmacy (Pharm P&D - 27/07'19), International Islamic University Chittagong, Bangladesh. 10 days before testing, animals were acclimatized to laboratory environment.

Acute toxicity study

Swiss albino mouse was used for the acute toxicity analysis under the E Walum, et al m ethod (Walum, 1998).

Elevated plus-maze (EPM) test

The process originally proposed by Handley et al. and was used with minor changes (Rabbani, Sajjadi, & Zarei, 2003) for this experiment.

Hole Board test

The segment test was carried out by using the previously described method. Y ozturk et al. (Chindo et al., 2003) with slight modifications.

Statistical Analysis

The statistical analysis was interpreted as mean ± standard (SEM) error, and analyzes have been carried out using GraphPad prism 5.2 (GraphPad Software, Inc., La Jolla, CA, USA). One way variance analysis (ANOVA) followed by Dunnett's analysis was used to determine the statistical significance of $*P<0.05$, $** P< 0.01$,$***P<0.001$.

Results

Preliminary phytochemical investigation

The MEAA screened for the qualitative determination of various phyto constituents. The present study assured the presence of protein, carbohydrates, flavonoids, saponins, phlobatannins, quinones, emodins and di-terpenes. The overall preliminary phytochemical results have been listed in **Table 1**.

Table 1. Secondary metabolites of MEAA (MEAA = methanol extract of *Amomum aromaticum* roxb. leaves)

SL No.	Phytochemical tests	Appearance
1	Carbohydrates (Molisch's test)	+
2	Cholesterol	-
3	Proteins	+
4	Alkaloids(Wagner's reagent)	_
5	Flavonoids (Alkaline reagent test)	++
6	Terpenoids (Salkowki's test)	-
7	Cardiac glycosides (Keller Kelliani's test)	-
8	Sterols (Liebermann-Burchard test)	-
9	Tannins (Braymer's test)	-
10	Saponins (Froth test)	+++
10.1	Saponins (Foam test)	+
11	Phlobatannins (Precipitate test)	++
12	Phenols (Ferric chloride test)	++
13	Quinones	+
14	Oxalate	-
15	Fatty acids	-
16	Anthocyanins	-
17	Leucoanthocyanins	-
18	Coumarins	+
19	Emodins	-
20	Diterpenes	+

In vitro anti-oxidant

DPPH Radical Scavenging Assay

The results of DPPH radical scavenging assays on plant extract and ascorbic acid and IC_{50} values of the samples are presented in Figure 1. IC_{50} of the standard and samples MEAA are 4.27 µg/mL and 4.27 µg/mL respectively.

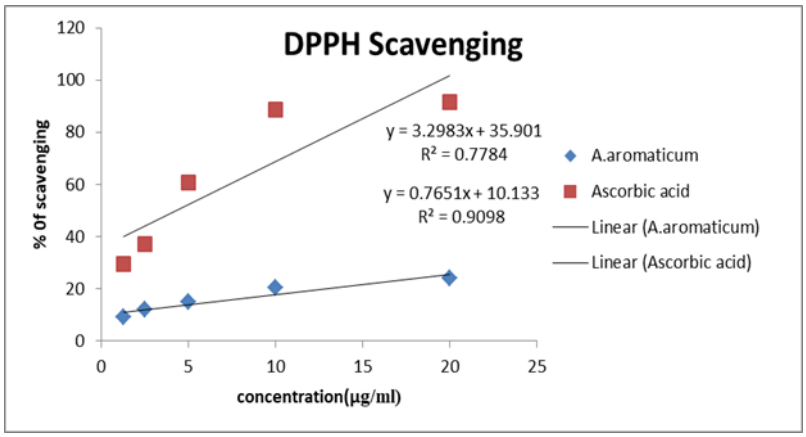

Figure 1. % of scavenging activity of ascorbic acid and MEAA at different concentration. MEAA = methanol extract of *Amomum aromaticum* roxb. leaves

Reducing Power Capacity

An important predictor on its possible antioxidant activities could be its reduction in the energy of the components of the plant extract. As shown in Figure 2, the reducing power of extract was increased by increasing sample concentration. The highest reducing power was observed 1.678 nm and 0.773 nm for ascorbic acid 1000 µg/mL and MEAA 1000 µg/mL respectively.

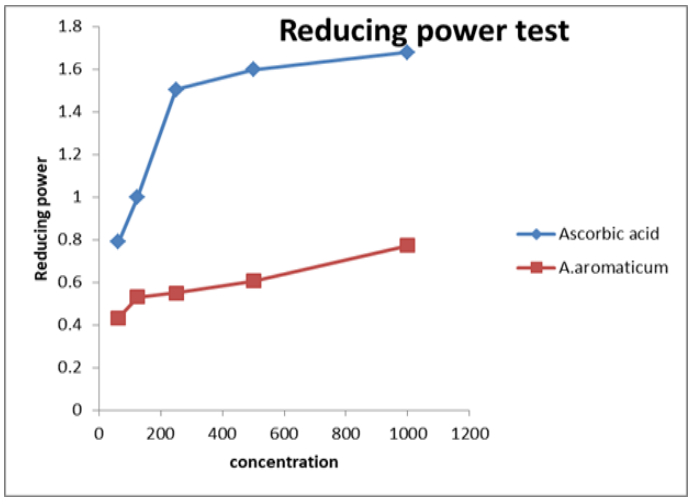

Figure 2. Reducing power of ascorbic acid and MEAA. MEAA = methanol extract of *Amomum aromaticum* roxb. leaves

Determination of total phenol content

In this analysis, the overall phenolic content of the sample evaluated as a standard curve gallic acid equivalent (y= 0.003x+0.033 and R2 = 0.998) was measured. The outcome was given as mg of the equivalent gallic acid. The average phenolic content of MEAA was 36.56 ± 1.02 mg. Descriptive phenolic content results has been shown in Figure 3.

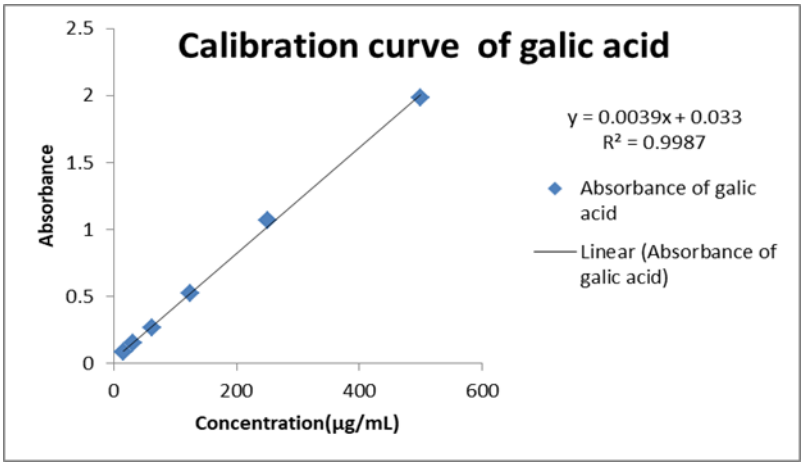

Figure 3. Calibration curve of Gallic acid for total phenol content determination

***In vivo* test**

Acute toxicity test

In vivo test Acute toxicity test, mice was survived and does not manifested any harmful signs at 1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 4000 mg/kg of doses.

Anxiogenic activity

EPM Test

In EPM test, besides control (positive and negative) animals were also treated with MEAA 200 and 400 (mg/kg, p;o, b,w.) showed decreases in their time spent in open arm. For MEAA 200 mg/kg, rodent spent in open arm for 41.10 ± 3.47 seconds and 258.90 ± 3.41 second in close arm. Again, for 400 mg/kg of MEAA, mice spent 34.83 ± 2.23 in open arm and 265.17 ± 2.23 second in close arms consequently where negative control (1% tween 80-10 mL/kg) and positive control (diazepam-1 mg/kg) attained 69.07 ± 2.02 seconds and 22.53 ± 2.94 seconds respectively in open arm and rest of the time they were in close arm. The average time spent in both open and close arm in EPM (Elevated pluz maze) test has been given in Figure 4.

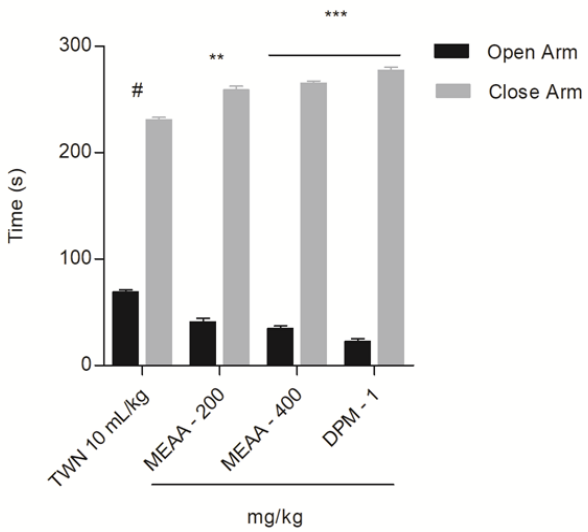

Figure 4. The effect of MEAA on Elevated Plus-Maze test in mice. Values are presented as mean ± SEM; One-way analysis of variance (ANOVA) was followed by Dunnett's test. *P <0.05, **P <0.01 and ***P <0.001 was considered as significant compared with the control, where # is designated as control. MEAA = methanol extract of *Amomum aromaticum* roxb. leaves

Hole Board test

In this test animals treated with MEAA at 200 and 400 (mg/kg, p.o, b,w) showed dose dependently decreased their dipping number, number of dipping decreased by both dose was 78.33 ± 6.642 and 47.00 ± 3.786 respectively where control (1% tween 80) showed the number of dipping for 99.33 ± 4.096 times. Similarly, animals treated with diazepam 1 mg/kg as standard, significantly attenuated the number of dipping, which was 17.67 ± 2.90. The total number of dipping by several test samples has been presented in Figure 5.

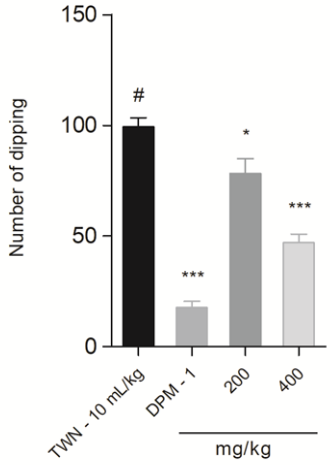

Figure 5. The effect of methanol extract of MEAA on Hole Board test in mice. Values are presented as mean ± SEM; One-way analysis of variance (ANOVA) was followed by Dunnett's test. *P <0.05, **P <0.01 and ***P <0.001 was considered as significant compared with the control, where # is designated as control. MEAA = methanol extract of *Amomum aromaticum* roxb. leaves

Discussion

Preliminary phytochemical, antioxidant and anxiogenic properties of the methanol extract of *Amouma romaticum* leaves have been addressed in this study. The phytochemical analysis of MEAA showed the existence of proteins, carbohydrates, flavonoids, saponins, phlobatanins, quinones, emodins and di-terpenes. There have been reports of substantial antioxidant activity of phenol and phenolic compounds like flavonoids. Tannins, for instance, are used as a substratum for determining antioxidant activity by means of iron deficit reduction, hydrogen limiting or complex interactions in essential proteins, such as enzymes DPPH (Dharmananda, 2003). Due to the formation of a non-radial DPPH-form by the interaction, the process is dependent on the reduction of methanolic DPPH solution in the presence of a hydrogen-donating antioxidant. The extract was capable of reducing the potent radical DPPH to di-phenyl-picryl-hydrazine with a yellow colour. The hydrogen-donning ability to minimize and decolorize 1,1-diphenyl-2-picrylhydrates has also been found to be cysteine, glutathione, ascorbial acid, tucopherol, polyhydroxyl fragrant substances (e.g., hydroquinone, pyrogallol or galllic acid). Gallic acid, a proven antioxidant, used as positive control, was significantly more scavenging activity (Kumaran, 2006). The scavenging ability of plant extract, ABTS+ radical has been demonstrated; this means that plant extract can be useful for the treatment of radical clinical damage particularly at a greater concentration (Wang et al., 1998). The reduction of the oxidized antioxidant molecule to regenerate the" active" reduced antioxidant is also a reaction path for electron donation. The subsequent antioxidant effect of ascorbic acid and of vitamin E on cell membranes where a-tocopheroxyl, a-tocopherol is regenerated in the existence of ascorbic acid is best demonstrated with its synergistic action (Burton, Wronska, Stone, Foster, & Ingold, 1990). The main contributors to several health problems are regarded

as Free Radicals such as diabetes mellitus, obesity, liver disease, renal failure and degenerative diseases owing to the inadequate natural protective mechanism of antioxidants (Parr & Bolwell, 2000). It is understood that in the Folin-Ciocalteu process, various phenolic substances have different responses. Except for two extreme cases (Chinese Galls and Catechu), the production of antioxidants also had a strong correlation with the overall phenolic content The outcomes revealed that the phenolic compounds significantly influenced the therapeutic herbs' antioxidant potential (Cai, Luo, Sun, & Corke, 2004). Anxiety is a psychiatric disorder which directly affects the standard of living of individuals and social relationships. In very severe symptoms great depression is taken into account. Drug therapy often utilizes pharmaceutical goods such as tri-cylindic antidepressants (TCAs), selective serotonin recapturing inhibitors (SSRIs), selective reversible monoamine oxidase A inhibitors (RIMAs) and common SNRRIs (Kumar, Lakshman, Velmurugan, Sridhar, & Gopisetty, 2014). Indirect GABA modulators are benzodiazepines that can enhance the GABAA receptor effect (40). Pentameric trans membrane receptors composed of 19 subtypes were identified to be GABAA receptors (α1–6, β1–3, β1–3, ć, ć, ć, α, μ, and ̈1–3) (Barua et al., 2009). In comparison to the consequences of anxiolytics, such as diazepam and chloro-diazepoxide, the findings of hole board studies gained no recognition from anxiogenics such as a benzodiazepine reverse agonist (File, Pellow, & Braestrup, 1985). The elevated plus-marsh was also very prone to the impact of a variety of anxiogenic compounds FG 7142 a /3-carbollin seen to cause significant anxiety on humans and animals exclusively decreased the proportion of open-arm entries and time spent on open-arm. Likewise the pyrasolone CGS 8216, which has several study protocols for its axiogenic behavior and the atypical Ro 5-4864 benzodiazepines, anxiogenic in the social interaction check and the Vogel test, have developed anxiogenic results (Pellow & File, 1984). Increases in the percentage of time on open arms indicate increases in anxieties and the strongest indicator of locomotive operation are numbers of close arms (Gonzalez & File, 1997). In the hole board study, shifts throughout head-dipping behavior that indicate the animals ' fear or anxiety status. MEAA has raised the head-dips in the current study while the locomotive in mice is not significantly changed. Diazepam therapy, a 5-HT1A receptor agonist, reversed the decline of head-dipping activity induced by severe restriction stress (Kamei, Matsunawa, Miyata, Tanaka, & Saitoh, 2004). Diazepam therapy, a 5-HT1A dopamine agonist, altered the decrease of the head dipping effects caused by severe restraint stress (Takeda, Tsuji, & Matsumiya, 1998). Phenol, Flavonoids, saponins, phlobatanins, quinones, carbohydrates, enzymes, di-terpenes, have been found in the phytochemical analysis for methanol extract of *Amomum aromaticum*. The antioxidant and anxiolytic capacity may be triggered by the presence of secondary metabolites.

Conclusion

The methanol extract of *Amomum aromaticum* exhibited modest antioxidant efficacy by inhibiting DPPH, reducing power and strong amount of phenolic content upon it was contrast with standard antioxidant substance which could be the modest source of drug for pain, inflammation, cancer and other oxidative degradation mediated disease managements . In both EPM and Hole board experiments, MEAA again found significant anxiety development which could be the potential source for the psychotic disorder managements. Activity guided isolation is therefore suggested to be studied in future.

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Conflict of Interest

No conflicts of interest are reported by the author.

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