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Fruit pulp and a seed as botanicals in dealing with pshychosomatic disorders in wistaralbino mouse model

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Abstract
Search of potential plant remedies and screening of petrochemicals serving as an anxiolytic and medication for psychosomatic disorders certainly calls attention in strategizing towards the meeting new normal demands of fortifying the health care equipment in post COVID 19 pandemic. Two non-conventional fleshy fruits, *Adansoniadigitata* L. and *Crescentiacujete* L., and a seed embryo extract of the legume *Caesalpiniabonduc*examined for their effects as anti-convulsing agent, muscle relaxant and mood stabilizer in the preclinical studies on Wistaralbino mice fetched interesting observations providing clues for further examination. It is observed that methanol fractions of the fruit pulp from the trees fared better than Bonducnut explants in granting relief from epileptic distress but withreference to the anxiolytic properties the seed legume offered best results. Consistent changes in source dependent and solvent specific variations monitored here show that the three studied selected botanicals can offer as effective inputs in neuropharmacological research.

Keywords: solvent extraction, grand mal epilepsy, extensor phase, depression, anxiolytic, actophotometer, EPM

Introduction

Novel corona virus with is variants traversing geopolitical boundaries beyond divides and distinction is now a huge unprecedented and nearly an untamed human disaster. This pandemic driven socio-economic crisis has created psychological stress and anxiety in individuals and community with its varied manifestations that a multi-pronged approach is needed for its combat. The viral onslaught has taken a toll on health and hygiene, resulted in joblessness alongside pressures of work and career aspirations for want clarity and sobriety in managing material resources and mind. Its woes exacerbated by growing frustration, deep anxiety and depression demands viable measures of mitigation (Sinha and Manna, 2020) [32]. Health-wise, stress involves complex biochemical, neural, and immunological mechanism triggering a disease state ranging from psychiatric disorder, cardiovascular diseases, hypertension, peptic ulcer, migraine, allergies, asthma, carcinoma, premature aging, rheumatic diseases and ulcerative colitis (Harter *et al.*, 2003; Kyrou and Tsigos, 2007) [19].

Pharmacotherapies with psychosomatic drugs find use in managing anxiety, stress and psychosomatic disorders. However, prolonged use of tranquilizers and psychosomatic drugs leads to autonomic and endocrine allergies, homotopic and serological side effects (Julien, 2013) [16]. Most anxiolytic agents like benzoidazepenes have adverse effects on memory. Psychosomatic drugs used to cure mental illness trigger many side effects ranging from sedation, hypotension, and extra pyramidal symptoms like dyslexia. On a comparative basis, natural remedies and crude drugs are of fewer side effects with least drug dependency (Jain *et al.*, 2014) [13]. Traditional Medicine involving phytotherapeutics in considered non-addictive as they address not merely the cure but the cause of the ailment (Rostain, 2011) [25]. Therefore the search biologics and natural plant drugs that mediate CNS in lieu of synthetic drugs prone to weaken vital body function demands special attention.

Psychosomatic Disorders

Psychology being a discourse that connects cognition, emotion and behavior calls for a study of complex and cohesive strategy wherein a reported ailment and its mitigation needs pharmacological breakthroughs with elaborate characterization of drugs (Martelle and Nader, 2008) [23]. This certainly is a daunting task as abnormalities in thought and behavior can originate beyond the realm of physiological functions. Imbalances in body and mind occur due to manifold reasons. Behavioral deviations can be classed under four major categories of psychiatric disorder namely Depression, Anxiety, Mania and Schizophrenia (Lieberman and First, 2018) [20]. *Depression* is a form of mental state characterized by a profound and persistent feeling of sadness or despair and/or a loss of interest in things that were once considered pleasurable. Disturbance in sleep, appetite, and mental processes are common accompaniments (Manroe and Harkness, 2012) [24]. The symptoms of depression include loss of appetite, sleep disturbances, irritability and in acute cases suicidal thoughts. Since synthetic medications prescribed for this evoke sharp changes in physiological or psychological functions with side effects, a search for bioremedybecomes a necessity (Sarris, 2013) [27].

Anxiety is the emotional condition that spans from mild apprehension and nervousness to intense fear and even

terror begetting tremor (Wittchen and Hoyer, 2001) [39]. It has been estimated that 2-4% of the population suffers from some form of anxiety disorder. Although anxiety is a common experience, if intense it could be debilitating (Rowa and Antony, 2008) [26]. Anxiolytics such as Propanediols, Barbiturates and benzodiazepines are advocated by psychiatrists for treatments. It has been shown that antipsychotic effect is due to an antagonistic action at dopamine receptors (Hasan*et al.*,2017). Drugs used in the context influence on histamine receptors in the cerebral cortex and thus cause sedation (Howes*et al.*, 2017). Disorders of mood, particularly depression, associated with a number of physical illnesses can be handled by Analgesics, Anti-depressants, Anti-hypertensive agents. *Epilepsy*is another psychosomatic illness connected with the disorder of brain. Epileptic seizure is a condition caused by neurobiological, cognitive, psychological and social consequences (Josephson and Jetty, 2017) [15].

By definition epilepsy involves the occurrence of seizures. An epileptic seizure is actually a transient occurrence of signs or symptoms of the abnormal excessive or synchronous neuronal activity (Scott *et al.*, 2017) [29]. The first-line treatment of epilepsy is the administration of an antiepileptic drug (AED). Selection of appropriate AED is based on diagnosis of the syndrome. First-line therapy for patients with focal seizures includes phenytoin, carbamazepine, valproteand Phenobarbital (Brodie, 2015). Substances of plant origin from several botanicals considered to be home remedies and indigenous medicine have come to the rescue in such cases as a part of community medicine (Abdolmaleki, 2020) [1]. Even though purified forms of drugs and synthetic allelopathic prescriptions are considered to be quicker and effective medications than crude extracts, natural complexes are desired as safe therapeutics, especially because they are free from adverse side effects (Cramaer*et al.*, 2010). It is in this context that the fruit pulp of two 'fleshy' fruit yielding trees namely Baobab (*Adansoniadigitata* L.) and Tree Calabash (*Crescentiacujete* L.) and seeds from a prickly straggling legume, Bonduc nut (*Caesalpiniabonduc*Roxb.) are taken up for a study.

Botanicals under consideration

Baobab Tree (*Adansoniadigitata* L.), locally referred as *Papparapuli* or *Annaipuli* (Tamil) owing to nature of the pulp in the hard shelled fruit is referred as therapeutic in the popular Siddha treatise *Gunapadam.* Ithas been pointed out that the fruit pulp can be administered as an antipyretic and can be helpful in controlling biliousness, vomiting, dysentery and excessive perspiration. Though the significance attached is variable from place to place the taxon is largely accepted to be a cure to bilious dyspepsia, toothache, gingivitis, putrid pestilential fever and is used as a emmenagogue, febrifuge, emollient, diaphoretic and prophylactic (Kabore *et al.*, 2011; Sundarambal*et al.*, 2015). Cauliflorous Tree Calabash (*Crescentiacujete* L., family Bignoniaceae), familiar for the hard shell of its fruit to ascetics and poor natives, being a large spherical fruit with a soft, acidic, photosensitive,fermentable pulp, though prone for fungal infestationgets reported with wider claims of medicinal potential. The sweet-sour fleshy pulp as such is a diuretic, antipyretic, aperient, febrifuge (Hetzel, 1993). Mesocarp of ripe fruit is made into a poultice and applied topically for headache. It has pointed out in TM that the leaves of *C. cujete* are used as emetic, purgative diaphoretic and can be used to treat bruised wounds, inflamed joints, sprains, ear aches and other ear complaints (Isalm*et al.*, 2019). Bonduc nut or Physic nut, *Caesalpiniabonduc* Roxb., much commonly known in Tamil medicine as *Kazharkai* or *Kazharchikai,* is the seed of a large armed liana with sharp hooks and hard yellow prickles. As a legume endowed with bipinnate compound leaves with long stipules and produces the typical Caesalpiniaceous showy yellow flowers forming is oblong and densely prickled leguminous pods the species assumes special medical significance. The soft moist pulp with seeds is reported to be a bitter, acrid, and astringent with thermogenic properties, used as anodyne, anti-inflammatory, digestive, stomachic, depurative, expectorant, contraceptive, antipyretic, anti-diabetic, rabefacient and an aphrodisiac besides providing for a liver tonic. Familiar to traditional medicinal practitioners, *Kazharchikai*isused both externally and internally to treat a number of ailments including infertility and canbe used as antiperiodic, antispasmodic, tonic, febrifuge and anthelmintic (Singh and Raghav, 2012; Shelar *et al.*, 2014; Lokonon *et a* l., 2021) [33, 30, 21].

Materials and Methods

1. Plant Material and Preparation of extracts

Fruits and seeds of the chosen plants that were collected and identified by taxonomist Dr.D.Stephan, Ph.D., Department of Botany, American College, Madurai-Tamilnadu, in the months of March and April.. After authentication and comparison with voucher specimen (No.AMC / HCN//28, 29, 30) leaf,dry and fresh fruit pulp from *Adansoniadigitata* and *Crescentiacujete* respectively, and the seed samples picked form the legume *Caesalpiniabonduc,* were shade dried and used as primary inputs for the preparation crude extracts. Shade dried material that had lost all moisture were ground well with a pestle and mortar that 200 g of the powder packed in Whatmann filter paper (grade no.1) sachets were subjected for hot extraction with Petroleum ether (60º-80º C), Chloroform (60º-62º C) and methanol (100º C) in a soxhlet apparatus.Allowing complete evaporation, the air dried residue in each case was dissolved in DMSO and treated as crude extract for preclinical evaluation.

2. *Experiments with animal models*

The present study involving extracts from the three plant sources on CNS based activity is evaluated through three specific methods as approved by experts in animal ethics committee of the KM college of Pharmacy, Madurai. Experiments were planned with different sets of test animals of similar age and weight. Separate animal groupings were made for determining therapeutic effects of extracts in each activity. *Tests performed on Central Nervous System (CNS) depressant activities were evaluated in comparison with the standard drug (Diazepam) with not less than six animals each in four different groups. Each animal used in the experiment weighed 20-30 g. The experiential design adopted in this study has been used earlier several times for evaluating the effects of hypnotics, sedatives, tranquilizers and anti-depressants (Vogel and Vogel, 2002).*

***Anticonvulsant activity* (Using Electroconvulsiometer)**

Epilepsy is the common devastating disorder to be evaluated first. Among the two laboratory models of studying induced

convulsions, the grandmal type and the petitmal type, the former was chosen for this study. Based on the principle of subjecting animals to supra maximal electric shock method, treated and untreated control animals, in this case the male albino Wistarmice were subjected to shock using pinna electrodes (150 mA alternating current) for 0.2 seconds stimulus duration. The resultant seizure in normal mouse showed a tonic phase of limb flexion around 2 sec followed by full tonic extension phase around 10 to 13 sec and few clonic jerks followed by the number of post tonic asphyxia(Cashin and Jackson, 1962).

Treatment protocol

Group-I: Served as normal control. Received 10ml/kg normal saline through oral administration

Group-II: Served as positive control with standard drug. (25mg/ kg Phenytoin Na., suspended with 1ml of 1% carboxymethyl cellulose fed as above in this specific trail)

Group-III: Served as treatment control 1. Received 100 mg/kg of petroleum ether extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above

Group-IV: Served as treatment control 2. Received 100 mg/kg of chloroform extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above

Group-V: Served as treatment control 3. Received 100 mg/kg of methanol extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above

Group-VI: Served as treatment control 4. Received 100 mg/kg, aqueous extract.

*Animals were grouped into the said six groups separately for each one of the plant extract and the entire experimental schedule was repeated thrice.

One hour after the administration of the saline/drug/ extract (as the case may be) the evaluation is commenced by subjecting the animals to electric shock. On using pinna electrode about 150mA current was delivered uniformly to all animal groups to stimulate the seizure. The percentage of animals which showed hard limb extension was evaluated.

CNS depressant functions **(Based on locomotor activity using Actophotometer)**

*Since substances that have allegiance to central nervous system influence the locomotor activity in man and animals, the change in the state of activity between the treated and untreated animals was taken as an index of CNS functions. The agility of animals determined as locomotor activity in the activity tray of Actophotometer which is a device that operates on the basis of animal crossing counts in the 10 light paths made using the photo electric cells, connected by a circuit to a counter. When a beam of light falling on the photocell is interrupted by the movement of the animal, a count is recorded and the number of such counts cumulatively evaluates the rapidity of the movement of animal which in turn reflects the extent of physical activity (*Dreitus, 1997).*Based on the consideration that sluggishness of the animal is proportional to the state of depression inferences were made in determining the efficacy of different treatments.*

Treatment Protocol*

Group-I: Served as normal control. Received 10ml/kg normal saline through oral administration

Group-II: Served as positive control with standard drug. (25mg/ kg Phenytoin Na., suspended with 1ml of 1% carboxymethyl cellulose fed as above in this specific trail)

Group-III: Served as treatment control 1. Received 100 mg/kg of petroleum ether extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above

Group- IV: Served as treatment control 2. Received 100 mg/kg of chloroform extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above

Group-V: Served as treatment control 3. Received 100 mg/kg of methanol extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above

Group-VI: Served as treatment control 4. Received 100 mg/kg, aqueous extract.

**Animals were grouped into the said six groups separately for each one of the plant extract and the entire experimental schedule was repeated thrice.*

*About 36*different albino mice of either sex picked at random, each weighing 20 to30 g were selected as test animals for this study. DMSO or the standard drug or the designated extract treatment was administered through intra peritoneal injection 10 minutes before the introduction of the animal in the activity chamber in the Actophotometer. Uniform time duration of 60 minutes was allowed for recording the data for every animal in each treatment. Starting from the control with six animals, readings were recorded in every batch and the mean value is calculated to mark the central tendency as well as deviations.

Anxiolytic activity **(Using Elevated Plus Maze)**

Elevated Plus Maze (EPM) is a special apparatus used to study the anxiolytic response of almost all types of anti-anxiety agents. The maze consists of 2 open arms (50cm x 10cm) crossed with 2 enclosed arms of the same dimensions with walls 40 cm height. The arms are connected to a central square, 10cm x 10cm to give the apparatus a plus sign appearance. The maze elevated 70cm above the floor in a dimly lit room fosters an ideal setting for the evaluation of this considered parameter. Rodents have a natural aversion for high and open spaces prefer enclosed arms that have a burrow-like ambience and hence their preference for open arm explorations checked in terms of number entries and occupancy in open arm is considered as the drug effect.

When put on the maze, animals experience an approach-avoidance conflict which is stronger in the open arms than closed arms. Untreated anxious animals that dare not to enter the open space confine themselves to the enclosed arm. Conversely one hour following drug administration, treated animals placed individually in the corner of an open arm and observed for a period of 10 minutes venture into open arm and show deviations in terms of (i) Percent performance for the first open arm entry, (ii) Number of entries in the open arm, and (iii) Duration of stay in the open arm. Increased exploratory activity is considered an indication of anxiolytic activity (Sonavane *et al*., 2002).

Treatment protocol*

Group-I: Served as normal control. Received 10ml/kg normal saline through oral administration

Group-II: Served as positive control with standard drug. (25mg/ kg Phenytoin Na., suspended with 1ml of 1% caboxymethyl cellulose fed as above in this specific trail)

Group-III: Served as treatment control 1. Received 100 mg/kg of petroleum ether extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above
Group-IV: Served as treatment control 2. Received 100 mg/kg of chloroform extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above
Group- V: Served as treatment control 3. Received 100 mg/kg of methanol extract of the selected plant extract dissolved with 0.5 ml DMSO, fed as above
Group-VI: Served as treatment control 4. Received 100 mg/kg,aqueous extract.

**Animals were grouped into the said six groups separately for each one of the plant extract and the entire experimental schedule was repeated thrice.*

Results

Plant extractions serve as potent neuropharmacological agents

Animal studies carried out using Wistaralbino mice indicate the utility of the selected species in dealing with CNS problems. Extracts prepared from the mesocarp of fruits from the two tree species, (*Adansoniadigitata* L., *Crescentiacujete* L.) and seed embryos of the legume (*Caesalpiniabonduc*exerted positive impacts of variable magnitude. Neuropharmacology as a study on the coordinated interaction of nerve, muscle and mind calls for a combined interpretation of related activities and hence animal behavior has to be observed in terms a variety of experiments. With CNS active ingredients, if there be any, extracts are bound to produce psychomotor slowing, emotional soothing and decreased anxiety. The responses of the animals assessed on basis of observations made on grandmal epilepsy, locomotor behavior in actophotometer and elevated plus maze experiments show that methanol and chloroform extracts were significant in offering good results.

Anticonvulsant activity

Grandmal epilepsy induced by electric shock toWistaralbino mice provided the basis for demonstrating the anti-convulsion effects of the plant extracts. It is found that intra peritoneal injection of extracts offered a quicker relief to the animals subjected to the electric shock. Animals subjected to 150 mA electric shock at the pinna regions of its ear typically showed the four different phases of recovery. Wistaralbino mouse receiving the mild shock survived and recovered by typically going through four phases of recovery before getting back to relaxed state. Of different phases namely Flexion (2-3 seconds), Extensor (13- 15 seconds), Clonus (3 seconds), and the Stuper (3 seconds), the extensor considered for comparison in this study clearly revealed the positive influence of plant extracts.

A test dose of 10 mg/kg decided upon after a preliminary study on LD 50 and ED 50 with the standard drug and extracts (not included in this report) showed that effective doses advocated based on the body weight of the animals were sufficient enough to evoke favorable responses. Drug and extract treatment helped the animals to recover from the impact of electric shock by shortening the extensor phase. The extensor phase which spanned for about 13 seconds in the untreated animal could be shortened significantly with the injection of the standard drug Phenytoin Na. Treated mice recouped in the shortest time period of 2.6 seconds. Even though plant extract did not exactly match or surpass the drug effect, it may be said that the overall results were encouraging. Extracts prepared out of fruit pulp of the arborescent species namely *Crescentiacujete* and *Adansoniadigitata* offered admirable results. As evident from the results shown in Table-1, *Adansonia* extracts differed marginally in their ability to speed up the recovery process depending on the nature of solvent used for elution. It may be noticed that the effectiveness of the extracts remained almost the same in the leaf and mesocarp samples (Figure-1). Efficiency rating made in terms of time saving went in favor of methanol extract although the crude extract could evoke only 70% success. It must be noted that this result in comparison with the data on standard drug is quite impressive.

Table 1: Neuropharmacological activities on animal models: Effects of plant extracts as anti-convulsion agents

S NO	*Adansoniadigitata*		*Crescentiacujete*		*Caesalpiniabonduc*	
Animal Class & Treatment type	**Duration of Extensor Phase (in sec)**	**Time saved on recovery**	**Duration of Extensor Phase (in sec)**	**Time saved on recovery**	**Duration of Extensor Phase (in sec)**	**Time saved on recovery**
Group I						
Control	13.25 +0.29	--	13.30 +0.29	--	13.25 +0.29	--
Group II						
Standard Drug Phneytoin Na. Positive control	2.4 +0.40	81.88 -	2.6 +0.40	80.45	2.4 +0.40	81.88
Group III						
PE	4.10 +0.30	69.05	4.9 +0.32	63.16	5.8 +0.38	55.63
Group IV						
CF	4.04+0.38	69.50	3.68+0.42	72.22	6.50+0.27	50.94
Group V						
ME	3.86+0.29	70.86	4.2+0.32	68.42	5.96+0.42	55.01
Group VI						
Methnaol Extract of leaf	3.92+0.32	70.41	4.08+0.39	69.20	7.16+0.18	45.96

All values expressed as mean + SEM
Values are found out by using One way Anova followed by Newman Kaul's multiple range test
a. Values are significantly different from normal control G1 at<0.01
b. Values are significantly different from normal control G2 at<0.01

Interestingly when extracts from fruit pulp of *Crescentia* were prepared with three solvents namely petroleum ether, chloroform andmethanol and tested for their effect in reducing the extensor phase, chloroform extracts produced best results (Figure-1). Fractions soluble in the solvent if injected sub-cutaneously to the test animals helped the Wistaralbino mouse to recover with 3.6 second after the administration of the extract. This in essence quickens the recovery process by about 72% when compared with that of the untreated control. As methanol extracts of fruit pulp reduced the time lag by 68%, petroleum ether fraction did the same by 63%. Interestingly leaf extracts of the same plant prepared using chloroform also exerted good positive results. On a comparative basis extracts prepared from the seed embryos of the medicinal legume *Caesalpiniabonduc*fell short of evoking a speedy recovery when compared with the tree species. In contrast to other plant samples that reeled out better responses in methanol (*Adansonia*) and chloroform (*Crescentia*), petroleum ether extracts provided better results of *Caesalpinia.* Leaf samples elicited relatively poor effect with the extensor phase of animals treated with the extracts extending up to as far as 7 seconds. Samples prepared from seed source were no better as they could only marginally improve upon this performance. *Caesalpinia* embryos could offer a relief to about 50% when compared with that of the untreated animal. Among the three taxa, *Adansoniadigitata* and *Crescentiacujete* samples were more convincing in their soothing effect on epileptic seizures.

Effects on locomotor functions

Another facet of CNS activity pertains to the results on animal behavior in the Actophotometer. It is found that the standard drug of the experiment Diazepam as well as the tested extracts slowed down locomotor functions in treated animals. When introduced in the activity tray of the instrument the untreated animals on an average were active enough to record an average score of 310 in their 10 minute stay in the activity tray of the instrument. Movement of animals monitored in 12 parallel light paths of light connected to the light sensors helped in assessing physical movements of the test animals. As the untreated animals mice were agile and aggressive in moving rapidly in all directions in the 1 x 1m chamber, animals treated with the standard drug Diazepam (4mg / kg of body weight) were sluggish and could manage only a mean score of 142 $\pm$ 1.5 indicating about 54% reduction in locomotor activity.

Though the animals injected with extracts showed no specific change in the pattern of movement they were emotionally quiet to offer consistent results in the repetitive trials. Table-2 shows the observations on mice dosed with *Adansonia*mesocarp extracts. It could be noticed that the movement of the animals slowed down when compared to that of the untreated animals indicating CNS depressant action. As observed in previous experiment, petroleum ether extract registered the lowest score. The cumulative reading of the 10 minute observation was 282 in the treatments which when compared with the standard and other solvent extracts suggests only a mild influence. In comparison the chloroform and ethanol fractions exerted much better influence to bring down the mobility of animals close to 25%. The effects compared using methanol based extractions revealed that the mesocarp extracts were better than the leaves.

Table 2: CNS depressant activities on animal models: Effects of plant extracts on locomotor activity

S NO	*Adansoniadigitata*			*Crescentiacujete*			*Ceasalpiniabonduc*		
Animal Class & Treatment type	**Activity score**	**Reduction (in %)**	**Relative efficiency (in %)**	**Activity score**	**Reduction (in %)**	**Relative efficiency (in %)**	**Activity score**	**Reduction (in %)**	**Relative efficiency (in %)**
Group I.									
Control	310+11.34	-	-	310+11.34	-	-	310+11.34	-	-
Group II									
Drug Diazepam Positive control	142+9.06	54	-	144+9.06	54	-	142+9.6	54	-
Group III									
PE	282+10.87	09	45	280+21.2	10	44	177+9.1	43	20
Group IV									
CF	235+18.6	24	30	288+14.9	07	47	168+6.8	46	15
Group V									
ME	239+13.06	23	31	203+17.2	35	19	162+9.5	48	12
Group VI									
Methnaol Extract of leaf	250+19.81	19	35	226+10.8	27	27	149+7.0	52	05

All values expressed as mean + SEM

Values are found out by using One way Anova followed by Newman Kaul's multiple range test

a. Values are significantly different from normal control G1 at<0.01

b. Values are significantly different from normal control G2 at<0.01

Almost a similar trend was evident in the experiments with *Crescentiacujete*fruit pulp extracts (Figure-2). It may be seen that chloroform and methanol were more effective in eluting active principles than petroleum ether. Mice injected with methanol based extracts showed about 35% effect when compared with the untreated animals. Though alcohol leaf samples could fetch only 27% effect the impact was higher when compared with results gathered from petroleum ether and chloroform extractions. As far as *Crescentiacujete* extracts are concerned chloroform as well as petroleum extracts showed far less effective results on locomotor functions than the alcoholic fraction extracted from the same source.

Interesting results on CNS depressant functions were in offing with *Caesalpiniabonduc* extracts. Seed embryo sources easily caused around 45% reduction in all solvent samples. It can be observed that as expected ethanol extracts were relatively more effective than the comparable isolates of other solvents. Methanol extracts prepared from seed embryo samples came closer to the standard drug Diazepam by restricting the animal movement to 6%. Methanol extracts made from the leaf samples showed an average of only 139 interruptions in the light path, which is in fact higher than the standard drug indicating there is scope for tapping this extract further.

Anxiolytic property of the plant extract

The overall results of the trials on anti-anxiety activity fetched more interesting insights on the behavioural pattern of animals. Experiments performed on the elevated plus maze helped to draw useful inferences on the psychopharmacological influence of plant extracts. Elevated maze is a simple apparatus used for studying the anxiolytic response of all most all types of anti-anxiety agents. The maze consists of 2 open arms (50cm x 10cm) crossed with 2 enclosed arms of the same dimensions with walls 40 cm high. The arms were connected to a central square 10 cm x 10 cm to give the apparatus a plus sign appearance. The maze was elevated 70 cm above the floor in a dimly lit room. Rodents have a natural aversion for high and open spaces and prefer enclosed arms, which have a burrow-like ambience and therefore spend greater amount of time in the enclosed arm.

When dozed with plant extracts which can allay fear, the animals experience an approach-avoidance conflict and hence visit the open arms at ease and move out from the enclosed arms. Owing to the superlative results observed with ethanol based extractions in the previous two experiments, only ethanol extracts of the selected plant species were taken for consideration in this particular trial. Mice injected with the blank saline made on average 13.05 and 9.66 entries in the closed and open arms respectively and stayed for about 107.16 seconds in the latter as it popped out. As against this animals injected with the standard drug Diazepam recorded a mean value of 7.14, 2.92 and 212.1 for the same said parameters (Table-3).

Table 3: Anxiolytic activities of plant extracts on animal models: Animal behavior on Elevated Plus Maze

S.No	GROUP	Number of entries in the Closed arm		Number of entries in the open arm		Time spent in the open arm in seconds	
		Number of entries	Percentage increase/ decrease	Number of entries	Percentage increase/ decrease	Time in seconds	Percentage increase/ decrease
1	Group I						
	Normal control	13.05+0.32	-	9.66+1.3	-	107.3+10.7	-
2	Group II						
	Positive control	7.14+0.73	-45.29	2.92+2.92	-69.77	212.7+15.7	-97.86
3	Group III						
	Extract 1 Adp M	9.4+0.95	-27.97 [31.65]	7.0+2.28	-27.54 [139.72]	115.0+11.3	7.18 [-45.93]
4	Group VI						
	Extract 2 Adl M	9.6+1.06	-26.44 [34.45]	6.6+2.05	-31.68 [126.3]	128.7+12.0	19.94 [-39.49]
5	Group V						
	Extract 3 Crp M	10.1+2.22	-22.61 [41.46]	8.2+1.85	-15.11 [180.82]	140.3+22.7	30.75 [-34.04]
6	Group VI						
	Extract 4 Crl M	10.9+2.95	-16.48 [52.66]	8.8+2.14	-8.90 [201.37]	132.3+19.3	23.3 [-37.8]
7	Group IX						
	Extract 7 Cbs M	7.8+0.65	-40.23 [9.24]	4.2+1.35	-56.52 [43.84]	190.3+8.13	77.53 [-1.05]
8	Group X						
	Extract 8 Cbl M	7.6+0.72	-41.76 [6.44]	3.8+1.11	-60.66 [30.14]	183.0+7.97	70.55 [-13.96]

All values expressed as mean + SEM Values are found out by using One way Anova followed by Newman Kaul's multiple range test Values are significantly different from normal control G1 at<0.01

a. Values are significantly different from normal control G2 at<0.01

b. Figures in square brackets indicate difference in %

Ethanol based extract treated animals showed enough variations in their responses which can be corroborated as a species impact. Generally test animals administered with extracts were at home spending their time in the closed arm and walked into the open arm without panic. This clearly showed that the extracts have evoked a favorable influence lessening anxiety in treated animals. As compared with the untreated animal that moved to the interior of the closed arm and made hesitant visits outside, mice administered with the drug as well as the extracts made casual and increased exploratory trips in the open arm. Of the six different samples, *Caesalpiniabonduc* served well as the source. Impressive results were available in both leaf and seed embryo extracts prepared from this genus (Figure-3).

In experiments conducted with *Adansonia*, animals dosed with mesocarp extracts made around 9.4 and 7 entries in the closed and open arms respectively with a stay of 115 seconds in the open arm. Results of the elevated plus maze with leaf samples from the same genus faired in almost on comparable scale. Animals administered with extracts from *Crescentiacujete* were relatively more relaxed in staying in open arm. Among the two sources of pulp *Crescentia* was relatively more effective in inducing useful changes. Nevertheless best results in offing with bonduc nut (*Caesalpiniabonduc*). Crude extracts from the legume imparted the best possible anti-anxiety effects only next to Diazepam (the standard drug). It was observed in extracts prepared from the seed embryos and leaf samples of *Caesalpiniabonduc*. With a mean of less than 8 and around 4 entries made in the closed and open arms of the plus maze, and mice injected with bonduc nut extracts were at their will in staying in both arms. It was interesting to see that the animals kept their cool in spending their time in the open arm and moved rather slowly and confidently reflecting the anti anxiety effects caused by the extract. The overall result

of bonduc nut samples was certainly the most closer in comparison with the standard drug.

Discussion

CNS and psychosomatic disorders manifested in terms of physical and mental illness recognized as Generalized Anxiety Disorder (GAD) in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM) remainsthe least studied and hence poorly understood health issue that confronts the civil society (Zhang *et al.,* 2020). The current surge in economic woes and rise socio-cultural crisis compounds the incidence of the chronic and episodic expression of the bunch of these ailments understood individually and specifically as epilepsy, depression, age-related dementia and debilitative mental disorders. Clinically addressed using anti-psychotic drugs and medications, the damages caused in the functional physiology and behavior of the affected individual in most cases are only managed and not addressed witha comprehensive and holistic care. Most prescribed ameliorants overlap symptoms of psychosis cast negative effects on attention, concentration, cognition and memory (Martelle, and Nader, 2008) [23]. Side effects caused by anxiolytic drugs and psychosomatic medications in market are not free of sedative-hypnotic, muscle relaxant and anticonvulsive effects. In the drive to look for natural remedies treating CNS, only a small number plant species have been reported to be scientifically evaluated. It goes without saying that very few of the active compounds have been isolated and identified (Jain *et al.,* 2014; Abdolmaleki, 2020) [13, 1]. Observations of this study alluding to neuropharmacological search present certain leads worth probing. In the three different set of trials, (i) studies on the study of locomotor functions in Actophotmeter, (ii) anti-anxiety effects on EPM, and (iii) the grandma epilepsy induced by electric shock using convulsiometer, Wistaralbino mouse evinced behavioral deviations suggesting positive results of varying magnitude.

Extracts offer positive mitigating response on Grandmal epilepsy

Observations made on the Central Nervous System (CNS) related functions show that the extracts of all three species are endowed the ability to influence psychosomatic functions. It was found in the study on grandmal epilepsy that extracts of *A. digitata* (ethanol extract of leaves) could help the animals get back to normalcy in less than 50% duration of the time normally taken to recover from electric shock. Though none could match the standard drug Phenytonin, substantial time saving was recorded in each one of the treatments (Table-1). An epileptic seizure recognized as the transient symptom of excessive synchronous neuronal activity in brain is probably calmed by the action of the plant extract in this experiment (Brodie, 2015). As reported in earlier studies ethanol extracts of seeds exerted the most favorable influence on neuromuscular coordination during epileptic seizures (Sucher and Carles, 2015) [36]. Fruit pulp extracts of the two trees had identical effects irrespective of the differences in source tissues and solvents used for extraction suggesting that there could be a common base for the observed therapeutic effect. In comparison, *C.bonduc* was relatively less effective than the mesocarp extracts of the other two samples (Fig-1). In *C. bonduc*, seed extracts were more effective than the eluents from leaves. As animals treated in this study recovered quickly from the grandmal epilepsy induced by electric shock without any drowsiness or lethargy, it may concluded that the six different extracts of the species used here are safe medicaments free from toxic effects.

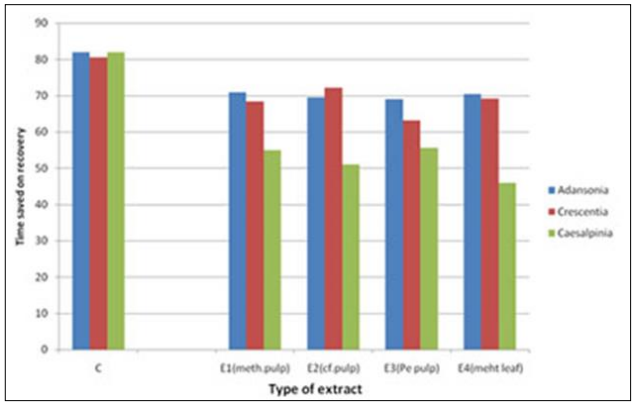

Fig 1: Comparative effects of the crude extracts of selected plant species as anti-convulsion agents

Observation on locomotor functions assessed by Actophotometer

In tests done on Digital Actophotometer, the extracts of *A. digitata* and *C. cujete*were seen to elicit good epileptic recovery performance, but in lower magnitude than extracts of bonduc nut. The locomotor behavior taken as an index to depict mood and mental calmness of the test animal was at its best with the drug Diazepam (Jayaraman*et al.,* 2010). As seen in Table-3 administration of Diazepam rapidly quietened the animals. In terms of the activity score, mice fed with Bonduc nut extracts gave the best results next only to the standard drug. Studies carried out in past have shown that phytochemical extracts have influenced locomotor activity by their antidepressant and skeletal muscle relaxant activity (Chakrabort*yet al.,* 2016). Observations made in the present study show that animals administered with extracts, especially seed embryo extracts of *C. bonduc* were steady and stable in their movement. Treatment with leaf extracts of bonducnut was as good as the standard drug (Fig-2).

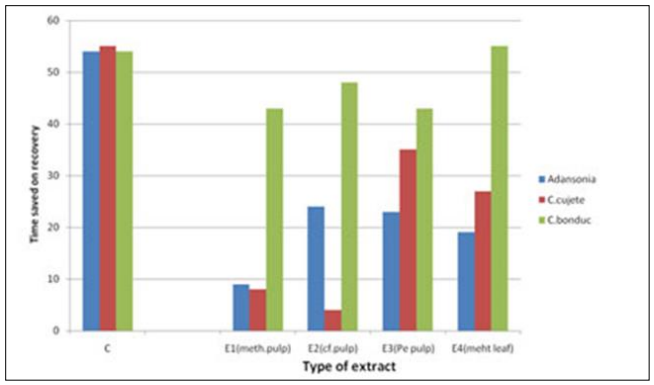

Fig 2: Comparative effects of the selected plant species on locomotor activity of albino mice in actophotometer

Chosen fruit pulp and seed extracts fetch anxiolytic properties

Elevated plus maze (EPM) being a simple and ideal tool to monitor anxiolytic properties of a drug expressed terms of behavioral response provides a direct measure for positive

inferences (Table-2).It has been shown in past investigations that anxiety disorders evoked by changes in mood, behavior, somatic function, and cognition can be gauged easily in the generally hyperactive rhodents by placing them on EPM and counting the number of entries in the open arm and the duration of occupancy there due to the soothing effects exerted by the drugs and extracts (Sonavane*et al.,* 2002). When compared to the untreated control, anxiety relief was more in extracts from seeds followed by the leaf of the legume and then the fruit pulp of tow tree sources. Among the assayed fruit pulp samples, *C. cujete* was more effective than *A.digitata*. Analyzing the source specific differences, it could be found that seed and mesocarpof the legume and tree calabash were more effective than the leaf source. Interestingly the trend is seen reversed with the somatic leaves of Boabobserving better than the fruit source.

Generally animals injected with the drug and extracts were seen less anxious and relaxed than the ones in untreated control suggesting a favored mood correction with least agitation. Analyzing similar responses using GABA, Deborah *et al*., (2005) [7] pointed out that the anxiolytic behavior can be correlated with the stimulation of glucocorticoid production and release in the adrenal complexIn investigating the impact of drugs on prelimbic (PL) subregion of medial prefrontal cortex implicated in anxiety regulation. Stern *et al*., (2010) [35] showed that mitigating the anxiety-related behavior may help and influence adoption at successive ventures of potentially threatening situation. Thus EPM experiment helps to concurrently investigate anxiety and the process of aversive learning and memory. It has been reported that CNS functions are more complex to be interpreted directly (Lundstrom *et al.,* 2017) [22]. Drugs that find their particular target sites in the nervous system induce widespread changes in the physiological and psychological functions (Kyrou and Tsigos, 2007) [19]. Psychoactive compounds and neural drugs are known to influence nerve, muscle and brain centers promoting CNS activity (Vogel and Vogel, 2002). Since anxiety dealt with the first generation major drugs including phenytoin, carbamazepine, valproateprovide relief only with side effects, results evinced by the crude extracts by passing this said constraint receives special significance (Fig-3). Based on the experiments of this study and the literature available on the activity of phytochemcial constituents in selected plants, CNS responses influenced by plant extracts can be recognized in discrete classes (Beaubrun, 2000; Jain *et al.,* 2014; Lundstrom*et al.,* 2017) [13, 22]. *Adansonia*which contained glycosoids and flavonoinds in its pulp and *Crescnetia*phytosterols and fixed oils in mesocarpcan be corroborated in assisting a speedy recovery of tested animals. Quite interestingly fruit extracts offered better results on seizure recovery than the seed legume. Reports published elsewhere point that the said substances along with the tannin could be implicated with the therapeutic action (Kumari and Jain, 2015) [18]. The muscle coordination and neuroprotective effects recorded in epileptic seizures in restricting the extensor phase of clonic-type seizures in grandmal epilepsy by the fruit pulp in *C. cujete* and *A. digitata*is seen comparable to the standard drug Phneytoin Na. Conversely the seed extracts detected with free amino acids, fixed oils, antroquinone glycosides and phytosterolsofbonduc nut that elicited less pronounced effects on epilepsy and the locomotor activity graded in actophotometer, offered excellent results on anxiolytic properties. Comparative results on EPM evaluation suggest*C. bonduc* seeds to be overwhelming. Ability of the methanol extracts of seed legume to quell the hyperactivenessof the test animal gelled well with the stabilized locomotor response of animal movement at actophotometer. Improved cognitive behavior of the treated animals in a potentially threatening situation at EPM help us to conclude that the medicinal legume more effective than the two tree members for CNS fortification. With the observations in conformity with the claims that leaves of Bonduc nut can be a phytotherapeuticwith no negative influence on memory and general health (Shukla *et al.,* 2009) [31], our present findingsshow that all three tested medicaments can be useful and effective remedies for psychotherapeutic ailments. Considering the importance and urgency of research needed on therapeutic intervention to deal neural disorders and psychosomatic problems (Satya*et al.,* 2018), results gathered now inspires further testing and follow-up.

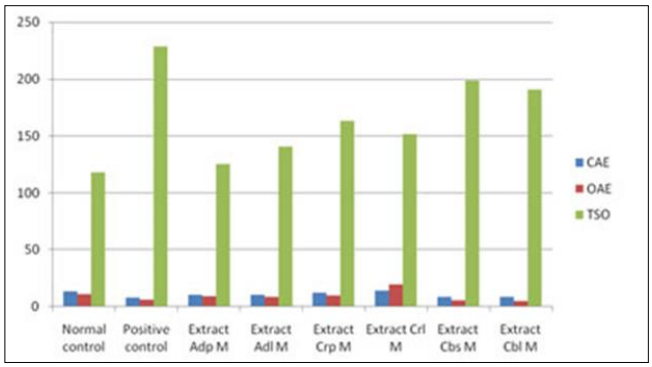

Fig 3: Anxiolytic properties of selected plant extracts o wistar albino mice in elevated plus maze experiments

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