Research Article

Phytochemical Profiling and Comparative Pharmacological Evaluation of Acetone and Methanol Extract of Combined *Murraya koenigii* and *Psidium guajava* Leaves for Antipsychotic Activity

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ABSTRACT

Psychotic disorders are severe mental illnesses characterized by hallucinations, delusions, and impaired cognitive function. Although conventional antipsychotic drugs are effective, their long-term use is associated with several adverse effects, highlighting the need for safer and more effective natural alternatives. The present study was designed to evaluate the phytochemical profile, antioxidant activity, and antipsychotic potential of *Murraya koenigii* and *Psidium guajava* leaf extracts. Fresh leaves were collected, authenticated, shade-dried, powdered, and extracted using the Soxhlet extraction method with acetone and methanol as solvents. The crude drugs were subjected to physicochemical evaluation, including ash values, moisture content, and extractive values. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, and tannins. Thin-layer chromatography (TLC) was performed to obtain phytochemical fingerprints of the extracts. Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was carried out using standard colorimetric methods. Antioxidant activity was assessed by the DPPH free radical scavenging assay, while acute oral toxicity was evaluated according to OECD Guideline 423 to determine the safe therapeutic dose. The antipsychotic activity of the extracts was investigated using the apomorphine-induced stereotypy model in Wistar rats, with chlorpromazine serving as the standard drug. Among the tested extracts, the acetone extract exhibited higher phenolic and flavonoid contents, stronger antioxidant activity, and a greater reduction in apomorphine-induced stereotypic behaviour than the methanol extract. The findings indicate that *Murraya koenigii* and *Psidium guajava* leaf extracts possess significant antioxidant and antipsychotic activities, suggesting their potential as promising natural therapeutic agents for the management of psychotic disorders.

Keywords: *Murraya koenigii*, *Psidium guajava*, phytochemicals, antioxidant activity, antipsychotic activity, TLC, flavonoids, phenolics.

INTRODUCTION

Psychosis is a mental condition marked by a separation from reality. Negative symptoms, delusions, hallucinations, significantly disturbed or aberrant motor behavior (including catatonia), and disordered cognition (speech) are the hallmarks of psychosis, according to the "Diagnostic and Statistical Manual of Mental Disorders." While psychosis can affect anyone at any age, older people should receive special attention about its causes, symptoms, and treatment. Two brain chemicals that may have a role in the emergence of psychosis are dopamine (DA) and serotonin.[1] The main focus of antipsychotic screening, treatment, and management is dopamine D2 receptor antagonism. Neurotransmitters including glutamate and serotonin (5-HT) are believed to be involved in schizophrenia in addition to dopamine (DA). Common antipsychotics, such haloperidol and chlorpromazine, mainly block the dopamine (D2) receptor in the limbic system. Olanzapine, clozapine, and risperidone are examples of atypical neuroleptics that inhibit the mesolimbic 5-HT2A receptor.[2]

The DPPH, a persistent free radical, is frequently used to evaluate antioxidant compounds' capacity to scavenge radicals. This method relies on the formation of the non-radical form DPPH-H, which causes DPPH in methanol solution to decrease when an antioxidant that donates hydrogen is present. This reaction results in a color shift from purple to yellow, according to spectrophotometric observations. The disappearance of the purple color is monitored at 517 nm. The ability to scavenge free radicals can be tested using 2,2 Diphenyl-1 picryl-hydrazyl.[3]

When apomorphine increases striatal dopaminergic activity, rats display stereotyped behavior.4 It has been reported that typical anti-psychotics prevent apomorphine-induced stereotypy by directly blocking the nigrostriatal dopamine receptor. Catalepsy is the term used to describe the long-term maintenance of an animal in an abnormal position imposed from the outside. Commonly used antipsychotics not only block the D2 receptor in the antagonistic mesolimbic region of the brain, but they also block the D2 receptor in the striatal region, which controls motor activity and results in cataleptic behavior in both humans and animals.[4]

MATERIALS AND METHODS

Plant profile: *Murraya koenigii* L

Often referred to as curry leaf, it is a member of the Rutaceae family. This little, fragrant tree is found across India and other South Asian nations. Bioactive components found in the leaves include flavonoids, phenolic compounds, carbazole alkaloids (murrayacine, girinimbine, and mahanimbine), and essential oils. The plant has long been used to treat a number of illnesses, such as neurological diseases, diabetes, inflammation, and digestive difficulties. According to recent pharmacological research, M. koenigii may have antipsychotic effects due to its neuroprotective,

antioxidant, and central nervous system-modulating qualities. Its capacity to lower oxidative stress and affect neurotransmitter systems implicated in psychotic illnesses is thought to be related to these actions.[5]

Psidium guajava L:

Guava, or Psidium guajava L. (family: Myrtaceae), is a tropical medicinal plant that is found in Asia, Africa, and South America. Bioactive components such flavonoids, tannins, triterpenoids, saponins, alkaloids, and essential oils may be found in the plant's leaves, fruits, bark, and roots. It has been revealed that quercetin and other flavonoids have important neuropharmacological effects. Guava leaves have been used in traditional medical systems to treat conditions of the central nervous system, diabetes, gastrointestinal tract, and inflammation. According to recent pharmacological studies, P. guajava leaf extracts may have antipsychotic, neuroprotective, and antioxidant properties because they can lower oxidative stress and alter neurotransmitter systems. These results provide credence to P. guajava's potential as a natural source for the creation of antipsychotic drugs.[6]

Figure 1: *Murraya koenigii*

Figure 2: Psidium guajava

1) Collection, Identification and Authentication of Plant Material:

At the Vidya Niketan College of Pharmacy in Lakhewadi, the leaves of Psidium guajava L. and *Murraya koenigii* L. are collected from Pune, Maharashtra, and identified. At Shankarrao Mohite Mahavidyalaya in Akluj, these plants were also confirmed. Dr. S.M. Satpute from the Head of the Botany Department.

2) Processing of crude drug:

Psidium guajava L. with *Murraya koenigii* L. Fresh leaves were pounded into a coarse powder after being dried in the shade. After passing through mesh number 14, the powder was stored for later use in an airtight container.[7]

3) Extraction of plant material:

For the extraction of *Murraya koenigii* L. and Psidium guajava L. leaf extract, selective solvents—acetone and methanol—were carefully chosen based on the extractive values and the kind of phytochemicals present in the drug. Selecting an Extraction Method The majority of researchers used Soxhlet extraction because the bulk of the chemical components of the plant extract are heat stable, according to a survey of the literature.[8]

a) Preparation of Acetone extract:

Powdered leaves of *Murraya koenigii* L. and Psidium guajava L. were used to create an acetone extract in a Soxhlet extractor using the standard method until a colorless solution appeared in a siphon tube. 248 grams of powdered husk and 1500 milliliters of acetone were used for the extraction. After the extraction procedure was complete, the solvent was cooled and dried. The extract was stored in an airtight container until it was required. The yield % of the extract was calculated.[9]

b) Preparation of Methanol extract:

In a Soxhlet extractor, powdered pod methanolic extract was prepared according to standard protocol until a colorless solution appeared in a siphon tube. 1500 milliliters of methanol and 245 grams of the powder were used for extraction. After the extraction procedure was complete, the extract was cooled and dried. The extract was stored in an airtight container until it was required. The yield % of the extract was calculated.[10]

4) Pharmacological Screening of Plant Extracts:

a) In-vitro Anti-oxidant activity

b) Safe dose calculation

c) Anti-psychotic activity

a) In-vitro Anti-oxidant activity: A material that may prevent other molecules from oxidizing is called an antioxidant. Oxidation is a chemical process that occurs when a material transfers electrons or hydrogen to an oxidizing agent. Free radicals can be produced by oxidation processes. These radicals can then initiate chain reactions. A cell may be damaged or perhaps die as a result of the chain reaction. By eliminating free radical intermediates, antioxidants stop these chain reactions and prevent further oxidation processes. The DPPH free radical scavenging test method was used to assess the extract's antioxidant properties.[11]

Preparation of test solution: *Murraya koenigii* L. and Psidium guajava L. leaf extracts (acetone and methanolic) were dissolved separately in 10 milliliters of methanol to

produce various quantities of 20, 40, 60, 80, and 100 micrograms per milliliter.[12]

Preparation of Standard solution: One milligram of the standard, quercetin, was dissolved in one milliliter of methanol to create various concentrations of 20, 40, 60, 80, and 100 micrograms per milliliter.[13]

Methods: 2,2 Diphenyl- 1 picryl-hydrazyl radical scavenging (DPPH) Activity:

Procedure: One milliliter of 0.1 mM DPPH solution in methanol, one milliliter of drug solution, and one milliliter of methanol make up the reaction mixture (3.0 ml). After vortexing the reaction mixture, it was allowed to sit at room temperature for half an hour in the dark. At 513 nm, the absorbance was measured. The control was a reaction mixture devoid of the test sample.[14]

b) Safe dose calculation:

When determining LD50, acute toxicity is taken into consideration. *Murraya koenigii* L. and Psidium guajava L. leaf extracts were administered at a safe dose of 2000 mg/kg in accordance with the OECD Guidelines for Acute Toxicity study: Class Method (423). According to the referenced study, graded dosages of extracts (up to a dose of 2000 mg/kg) had no discernible effects on the animals' alertness, motor activity, respiration, restlessness, diarrhea, convulsions, coma, or appearance. Psidium guajava L. with *Murraya koenigii* L. Rats exposed to up to 2000 mg/kg of leaves did not die, indicating that leaves are safe for biological research. Therefore, 400 mg/kg was determined to be the maximal therapeutic experimental dosage.[15]

Animal used: Wistar rats weighing between 150 and 200 grams, regardless of gender

Test group: seven animal groups were created for the study. There are six rats in each group.

Administration method: oral [16]

Housing condition: Each of the six groups of animals was housed in a cage with a temperature control of 22±2 degrees Celsius. Each animal was given a normal diet (golden feed, New Delhi) and a steady supply of water. The animals were divided into six treatment groups, each containing three rats, at random.[17]

IAEC Approval: 150–200 g Wistar rats of either sex were utilized in this study. The animal house at Vidya Niketan College of Pharmacy in Lakhewadi, where the experimental animals were housed under typical laboratory conditions, was authorized by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Protocol. The animals were fed a commercial pellet diet, had full access to water, and were kept at a controlled temperature of 24 ± 2°C with 12-hour light/dark cycles. Each animal had at least a week to acclimate to the lab environment before the trial began. Committee on Animal Ethics in Institutions.[18]

c) In-vivo studies:

Animal grouping

Despite being a dopamine receptor agonist, apomorphine's dopaminergic activity results in compulsive stereotypical behavior in rats. Stereotypical behaviors include licking, sniffing, and standing (rising) repeatedly. The phrase "compulsive behavior" describes the mindless actions of an animal. The aberrant behavior associated with schizophrenia is caused by excessive dopamine neuronal activity in the limbic system. Excision antipsychotic model The excision psychotic mode was employed in the seven-day trial. The rats exhibited stereotyped responses such sniffing, rearing, and licking after being sedated with chloroform and treated with chlorpromazine at doses of 3 mg/kg for 1, 4, and 7 days prior to the administration of apomorphine (induced model). When apomorphine increases striatal dopaminergic activity, rats display stereotyped behavior.[19]

Animals will be divided into following grouping each containing 6 animals

Table 1: Animals grouping

GROUP	TREATMENT	DOSE & ROUTE
Group I	Normal Control	Vehicle (0.5% CMC, p.o.)
Group II	Disease Control	Apomorphine (2 mg/kg, s.c.)
Group III	Standard	Chlorpromazine (3 mg/kg, i.p.) + Apomorphine (2 mg/kg, s.c.)
Group IV	Acetone Extract (Low Dose)	200 mg/kg (p.o.) + Apomorphine (2 mg/kg, s.c.)
Group V	Acetone Extract (High Dose)	400 mg/kg (p.o.) + Apomorphine (2 mg/kg, s.c.)
Group VI	Methanol Extract (Low Dose)	200 mg/kg (p.o.) + Apomorphine (2 mg/kg, s.c.)
Group VII	Methanol Extract (High Dose)	400 mg/kg (p.o.) + Apomorphine (2 mg/kg, s.c.)

Inhibition of Apo morphine stereotypy in rats

Procedure: Another group of male rats (n = 5–6) received apomorphine HCl (1.5 mg/kg, s.c.). Every rat was placed in a separate cage. Ten minutes later, apomorphine-induced stereotyped behaviors were seen, including persistent licking, sniffing, and rearing. The animals were administered apomorphine 30 or 60 minutes after receiving either a vehicle, a test medication, or a reference chemical

(chlorpromazine [3 mg/kg, i.p.]), and stereotyped behavior was observed as previously described. Another group of male rats (n = 5–6) will receive apo morphine HCL (1.5 mg/kg s.c.). Every rat was placed in a separate cage.[20]

Interpretation: a decrease in or total reversal of stereotypical behavior Apomorphine-induced anti-psychotic effects are symptomatic of test drug therapy.

Drugs: Chlorpromazine (normal drug), apomorphine (induced drug).

Sniffing, rearing, and licking are evaluated parameters.[21]

RESULT AND DISCUSSION

Pharmacological evaluation of *Murraya koenigii L. and Psidium guajava L leaves* extracts

In-Vitro Antioxidant activity

The percentage inhibition of acetone and methanolic of *Murraya koenigii* L. and Psidium guajava L. leaves at 513 nm was measured using the DPPH Scavenging Assay technique at various concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml, respectively. Ascorbic acid was used as a reference standard to compare the results, and both extracts demonstrated a very strong percentage inhibition that was comparable to the reference standard. *Murraya koenigii* L. and Psidium guajava L. leaves were extracted using acetone. has the greatest percentage of inhibitory activity (76.70% at a dose of 100µg/ml).

In vivo Antipsychotic activity:

According to the observations, by Day 7, the TCAE 200 mg/kg group performed similarly to Chlorpromazine, as seen by the non-significant difference (#) between their outcomes. Overall, the acetone extract showed more action than the methanolic extract, indicating that the neuroprotective effect may be mainly caused by non-polar phytoconstituents. These results confirm the anti-psychotic properties of the leaves of Psidium guajava L. and *Murraya koenigii* L. and promote further research into its pharmaceutical uses.

Table 2: % inhibition of acetone and methanolic leaves extracts

	Std. Ascorbic acid			Acetone Extract		Methanol Extract	
Sr. No	**Conc. (µg/ml)**	**Absorbance**	**% Inhibition**	**Absorbance**	**% Inhibition**	**Absorbance**	**% Inhibition**
1.	20	0.482 ± 0.02	43.4 ± 0.10	0.483±0.0017	41.02	0.474±0.0014	42.12
2.	40	0.374 ± 0.001	55.20± 0.18	0.315±0.0011	61.53	0.361±0.0008	55.92
3.	60	0.280 ± 0.001	64.56± 0.18	0.286±0.0011	65.07	0.296±0.0008	63.85
4.	50	0.195 ± 0.001	74.2o± 0.16	0.234±0.0011	71.42	0.242±0.0017	70.45
5.	100	0.153 ± 0.002	85.90± 0.11	0.207±0.0005	76.70	0.209±0.0014	73.48

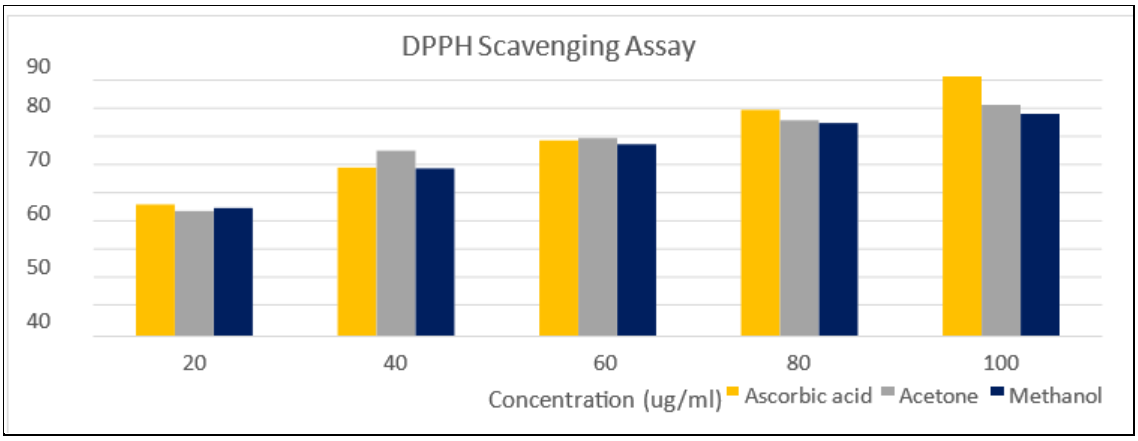

Figure 3: DPPH Scavenging Activity *leaves* extracts

Table 3: Stereotypy behaviour-Sniffing

Treatment group	**Inhibition of Apomorphine stereotypy in rats (Stereotypy behaviour -Sniffing)**		
	Day 1	**Day 4**	**Day 7**
Normal Control	0±0.000	0±0.000	0±0.000
Disease Control	14.5±0.22	16.5±0.2	13.8±0.30
Standard	5.33±0.21**	5.33±0.21**	5.32±0.21**
Acetone Extract (Low Dose)	6.66±0.21**	6.63±0.21**	6.33±0.21**
Acetone Extract (High Dose)	5.33±0.21**	5.34±0.21**	5.34±0.22**#
Methanol Extract (Low Dose)	7.5±0.22**	7.3±0.21**	7.8±0.16**
Methanol Extract (High Dose)	7.8±0.30**	6.33±0.22**	6.8±0.16**

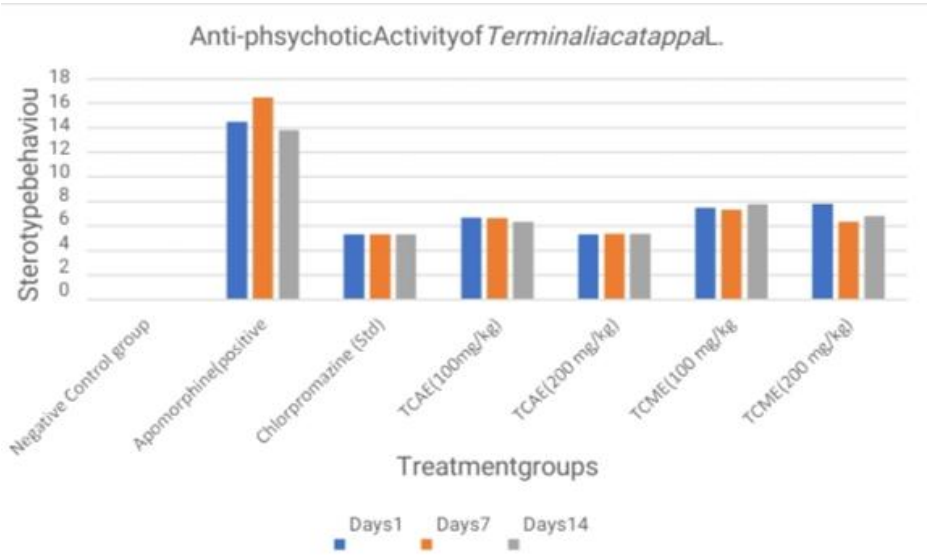

Figure 4: Effect of leaves extracts on Anti-psychotic activity

CONCLUSION

The present study demonstrated that the combined leaf extracts of *Murraya koenigii* and Psidium guajava possess promising phytochemical and pharmacological properties. Preliminary phytochemical screening and TLC fingerprinting confirmed the presence of important bioactive constituents, including alkaloids, flavonoids, phenolic compounds, tannins, and steroids. Quantitative analysis revealed that the acetone extract contained higher total phenolic and flavonoid contents than the methanolic extract, which was positively correlated with its superior antioxidant activity in the DPPH free radical scavenging assay.

Furthermore, the acetone extract exhibited significant antipsychotic activity in the apomorphine-induced stereotypy model, producing a marked reduction in stereotypic behaviour comparable to the standard drug chlorpromazine. These findings suggest that the neuroprotective and antipsychotic effects of the extract may be attributed to its high concentration of phenolic and flavonoid compounds with potent antioxidant properties.

Overall, the study provides scientific evidence supporting the therapeutic potential of the combined acetone extract of *Murraya koenigii* and Psidium guajava leaves as a natural source of antioxidant and antipsychotic agents. However, further studies involving bioactive compound isolation, mechanistic investigations, and long-term safety and clinical evaluations are required to validate its potential for future pharmaceutical development.

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