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Comparative Antiemetic Study of Fruit, Leaves and Bark Extract of Cordia Gharaf and Cordia Myxa via Dopaminergic Pathway: *in-vivo* Study

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Abstract: Vomiting, often associated with various ailments and as a side effect of treatments like chemotherapy, remains a challenging symptom to manage. This study aimed to evaluate the antiemetic properties of ethanolic extracts derived from the bark, fruit, and leaves of *Cordia gharaf* and *Cordia myxa*. Using a copper sulphate-induced chick emesis model with young chicks aged 7-10 days, we assessed the reduction in the number of retches as a measure of antiemetic efficacy. Extracts were administered at a dose of 150mg/kg, with Chlorpromazine (150mg/kg) and Domperidone (100mg/kg) serving as reference standards. Notably, the ethanolic extracts from *C. gharaf* bark, leaves, and *C. myxa* fruit demonstrated significant antiemetic effects compared to the standard groups. Phytochemical analysis revealed the presence of flavonoids and alkaloids in these extracts, suggesting their potential contribution to the observed antiemetic activity. These findings highlight the prospective role of these extracts as natural antiemetic remedies, warranting further exploration in future research endeavors.

Keywords: Copper sulphate, Cordia gharaf, Cordia myxa, Retching, Vomiting.

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Introduction

Historically, the utilization of plants and its phytoconstituents with medicinal principles have been in medical practice for many centuries. Plants have been broadly utilized for the prophylaxis and remedy to address various health issues. Estimated 25% prescribed drugs and 121 active agents used are derivatives of plants. According to World Health Organization list, out of 252 essential medicines 11% are plants procured. Primarily, 80% population of Asia and Africa depend on natural medication for treatment [1].

The physiological condition of human bodies changes all over life because of hormonal and mechanical effects. These effects will progress in different illness and symptoms. The most common symptom associated with the human body is vomiting. Generally, Emesis is ejection of gastric substance of the digestive tract through mouth. It is a sign of widespread disorders like gastritis, overexposure to radiations, foodborne illness, detrimental repercussions of drugs like chemotherapy and opioids etc. The chemoreceptor trigger zone acts as a boost for vomiting. Emesis management is chosen conforming to the pathophysiology of emesis [2]. Domperidone is a potent antiemetic agent that stimulates the motility of bowel and stomach resulting in treatment

of vomiting [3]. Similarly, Chlorpromazine is also potent antiemetic, especially vomiting induced by chemotherapy in pediatric oncology[4]. However, domperidone and chlorpromazine have side effects like sedation, arrhythmia, polydipsia by chlorpromazine [5] and cardiotoxicity especially in Parkinson's disease patients by domperidone [6] also acute dystonia rarely reported side effect by domperidone [7].

Despite having considerable clinical significance as antiemetic agents that's why safety apprehensions of these antiemetic agents have triggered the finding and development of the novel antiemesis agents with less side effects. In the past numerous natural substances have been reported to have antiemetic activity like *Zingiber officinalis* Roscoe. (ginger) is one of the potent natural antiemetic agent due to the presence of biomarkers like gingerols, paradol, shagaols and zingerone [8]. As well as natural products are frequently consumed alternative medicine throughout the world [9]. So, there is a dire need to discover antiemetic agents with potency like ginger and which are economical as well.

Genus *Cordia* has become popular due to its consumption as traditional medicine and have various phytochemicals reported which are found to be biologically and pharmacologically active and led to aid in different ailments [10]. *Cordia gharaf*, a small tree belonging to the family Boraginaceae that grows mostly in tropical and temperate regions. It has significant medicinal importance. *Cordia gharaf* contain several phytochemicals like alkaloids, flavonoids, glycosides, steroids and tannins. Reported pharmacological activities are antiseptic, astringent, anti-inflammatory, antidiabetic, antidiarrheal and antileprotic [11]. *Cordia myxa* belonging to the family Boraginaceae is a dioecious shrub or small tree found in temperate and tropical Asia, also in Mediterranean regions. Boraginaceae contain several phytochemicals like alkaloids, flavonoids, saponins, tannins, glycoside, steroid and coumarins. Reported pharmacological activities in different parts of the *Cordia myxa* are analgesic, anti-inflammatory, antimicrobial, antiparasitic, cardiovascular, gastrointestinal, immunomodulatory, insecticidal, and protective effects [12-14].

Previously, no research has been conducted on *Cordia gharaf* and *Cordia myxa* for anti-emetic effect, hence the current research emphases on evaluating the anti-emetic capability of fruit, leaves and barks of *Cordia gharaf* and *Cordia myxa.*

Materials and Methods

Collection of Plant Material

Fresh plant of *Cordia gharaf* and *Cordia myxa* having leaves, flowers, fruits and bark were collected from the University of Karachi, Karachi, Pakistan and validated by the Department of Pharmacognosy, University of Karachi with herbarium voucher number CG-0322 (*C. gharaf*) CM-0522 (*C. myxa*).

Figure 1. *Cordia gharaf*Ehrenb. Ex. Asch

Figure 2. *Cordia myxa* L.

Preparation of Ethanolic Extract

1kg portions of the bark, fruits and leaves of *Cordia gharaf* and *Cordia myxa* were washed separately with distilled water and afterwards drying were subjected to grinding and macerated in

ethanol (1.5 liters each) exactly for 07 days. Macerated solvents were strained out by means of muslin cloth to eradicate debris and again strained via Whatman filter paper No. 1. Afterwards, the unwanted solvents were vaporized individually through Rotary evaporator R-3, Vacuum pump 700+Nanometer, Chiller F-100 to achieve concentrated extracts. Extracts were kept at room temperature of 23-25 ± 2ºC in an airtight amber glass container.

Chemicals
Ethanol (Merck Chemical Co, Darmastadt Germany), Copper sulphate (Shahsons (PVT) Ltd-Karachi, Pakistan. Sartan (chlorpromazine HCl) from Platinum Pharmaceuticals (Pvt.) Ltd. and Motilium (domperidone) from Aspin Pharma were obtained.

Animals
Chicks with good physical conditions of both sexes, 7-10 days old having weight between 40g – 49 g were procured big bird hatchery PVT LTD. Karachi Pakistan. Then chicks were kept in the animal center of Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. The appropriate research environment was provided to the chicks in the laboratory at an ambient room temperature of 12 hours dark and light cycles where food and water is accessible. Three days later chicks were kept away from food and water to attain fasting for about 24 hours and the research was conducted afterwards. Animal testing during the trial was proficient in compliance with the requirements stated by Hubrecht and Kirkwood 2010 [15] also the research was sanctioned by the institutional board of the University of Karachi.

Acute Toxicity Studies
According to OECD protocols, toxicity of *Cordia gharaf* and *Cordia myxa* extracts was observed at different doses of (50 mg/kg, 100 mg/kg and 150 mg/kg) p.o to chicks (N=7). The behavioral changes and mortality rate of animals were observed for 48 hours for 2 weeks [16].

Drug Administration
Animals included in testing were bifurcate into five groups respectively containing (N=7) chicks in every group. Group I was categorized as negative control (copper sulfate 50mg/kg), Group II, III and IV were characterized as Treated I, Treated II and Treated III (Ethanol extracts of fruits, leaves and bark 150mg/kg) of *C. gharaf*. Group IV, V and VI were marked as Treated I, Treated II and Treated III (Ethanol extracts of fruits, leaves and bark 150mg/kg) of *C. myxa*. Group VII and VII were used as standard (Chlorpromazine and Domperidone 150 mg/kg b.w) [17]. Both the extracts were reconstituted in distilled water to get the ultimate concentration. An oral gavage dosing technique was applied to all groups of chicks for administration of an extract dose.

Determination of the Antiemetic Effect
The antiemetic activity was assessed through copper sulphate-induced chick emesis model. Every animal was confined in a substantial plastic cage and left for at least 10 minutes. The ethanolic extracts of *C. gharaf* and *C. myxa* fruit, leaves and bark were reconstituted at a dose of 150 mg/kg body weight in distilled water. Copper sulfate (50 mg/kg) was administered to a negative control group, whereas positive control groups were first administered with standards like (chlorpromazine and domperidone) and treated (ethanolic extracts of *C. gharaf* and *C. myxa* fruit, leaves and bark) at the dose of 150 mg/kg sequentially. After almost 10 minutes of dose administration, the chicks were subsequently administered copper sulfate 50mg/kg orally and the number of retches were observed for next 10 minutes, [18, 19] hereafter percent inhibition was determined by the following formula:
Inhibition (%) = (A-B/A) x 100
Where, A = Frequency of retching in the negative control group; and B = Frequency of retching in

the test group.

Phytochemical Analysis
Phytochemical investigation of phytoconstituents, like alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins and tannins, etc present in plant extracts were carried out by standard procedure [20, 21].

Statistical Analysis
The data of no. of retches in study for antiemetic effect was expressed as Mean S.EM. Un paired students' *t*-test was performed for statistical calculations.

Result
Acute Toxicity Studies
All extracts of *Cordia gharaf* and *Cordia myxa* were found to be safe at the dose of 150mg/kg in chicks p.o. body weight. No signs of toxicity of extracts at 150mg/kg dose were observed and no mortality, stress, loss of weight, paralysis, tremor, lethargy and other adverse effects were recorded.

Antiemetic Activity
As stated in Table 1. when both the standards and treated groups were compared with negative control, a noteworthy decrease in number of retches in treated groups and standard II (domperidone) was observed, also showed a significant depletion in frequency of retches as compared to negative control.
Table 1. The antiemetic effect of extracts of *Cordia gharaf* and *Cordia myxa.*

Treatment	Dose (mg/kg) p.o	No. of Retches (Mean ± SEM)	Inhibition (%)
Control	---	68.50±3.20	---
CPZ	150	46.62±3.84 *	31.94
DM	100	12.43±0.37**	81.85
CGB	150	6.47±0.12*	90.55
CGF	150	27.90±0.22**	59.27
CGL	150	18.70±0.58**	72.70
CMB	150	30.76±0.48**	55.09
CMF	150	11.15±0.57**	83.72
CML	150	29.83±1.10**	56.45

Note: CPZ = Chlorpromazine ; DM = Domperidone ; CGB = *Cordia gharaf* bark ; CGF = *Cordia gharaf* fruit; CGL = *Cordia gharaf* leaves; CMB= *Cordia myxa* bark ; CMF = *Cordia myxa* fruit; CML= *Cordia myxa* leaves ; N=7 ; *P< 0.05, *P< 0.001 vs. control showing significant value(s) using un paired students' *t*-test.

When chlorpromazine (standard I) was compared with domperidone (standard II) it was seen that Domperidone significantly reduced the number of retches (81.85 % inhibition) as compared to Chlorpromazine (31.94 % inhibition). In treated groups *C. gharaf* bark, *C. gharaf* leaves and *C. myxa* fruit extract showed significant reduction in number of retches (90.55, 72.70 and 83.72 % inhibition) respectively. Figure. 3 shows the percent emesis inhibition and significance of extracts of *Cordia gharaf* and *Cordia myxa* by Mean in No. of Retches is shown in Figure. 4.

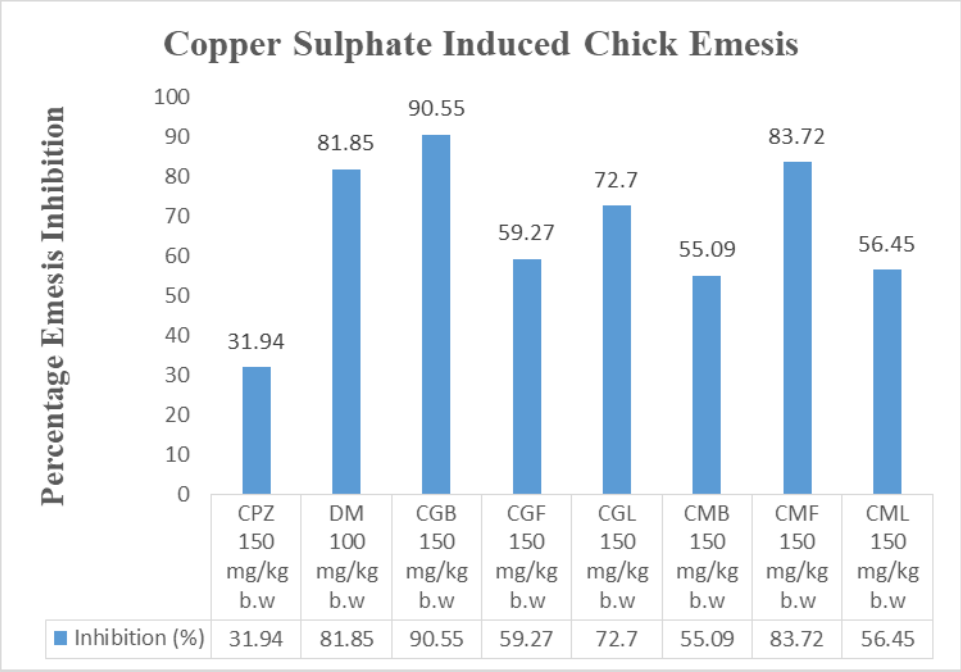

Figure 3. Percent Emesis inhibition of extracts of *Cordia gharaf* and *Cordia myxa.*

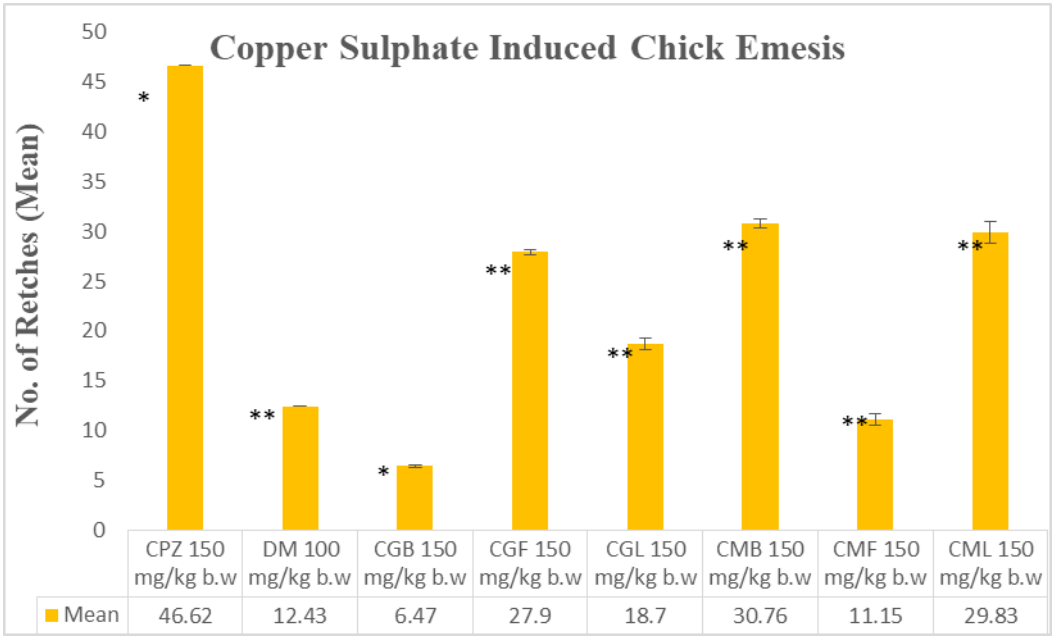

Figure 4. Comparative analysis of treatment Effects on mean number of retches

Phytochemical Analysis

In Table 2. phytochemical studies have been shown for the presence of phytoconstituents like flavonoids and alkaloids.

Table 2. Qualitative phytochemical analysis of *Cordia gharaf* and *Cordia myxa*.

Plant Part	***Alkaloids***	***Anthraquinones***	***Flavonoids***	***Saponins***	***Tannins***	***Triterpenoids***

CGB	+	-	+	-	+	+
CGF	-	-	+	-	-	-
CGL	+	-	+	-	-	-
CMB	+	-	-	-	-	+
CMF	+	-	+	-	+	-
CML	+	-	+	-	+	+

Note: CGB= *Cordiagharaf* bark ; CGF = *Cordia gharaf* fruit; CGL = *Cordia gharaf* leaves; CMB= *Cordia myxa* bark ; CMF = *Cordia myxa* fruit; CML= *Cordia myxa* leaves; + (positive); -(Negative).

Discussion

The safety profile of the *Cordia gharaf* and *Cordia myxa* extracts at a dose of 150mg/kg in chicks was rigorously evaluated both pre- and post-experimentally. Prior to administration, thorough examination and observation revealed no indications of toxicity. Subsequently, throughout the experimental period, including post-administration, no adverse effects such as mortality, stress, weight loss, paralysis, tremors, or lethargy were observed in the chicks. These comprehensive pre- and post-experimental assessments provide compelling evidence supporting the absence of toxicity associated with the administration of the extracts at the specified dose, affirming their safety for use in avian models.

The dose selection of 150mg/kg for the administration of *Cordia gharaf* and *Cordia myxa* extracts in the chick antiemetic study was based on several considerations. Firstly, this dose was chosen to align with standard practices in pharmacological research, aiming for a dose that would elicit measurable effects while minimizing the risk of adverse reactions. Additionally, previous literature on similar plant extracts and antiemetic agents often utilized doses within this range, allowing for comparability and reference to existing studies. Moreover, preliminary dose-ranging studies might have been conducted to determine a dose that demonstrates efficacy without inducing toxicity. Considering these factors, the dose of 150mg/kg was deemed appropriate for evaluating the antiemetic effects of the extracts in the chick model, balancing efficacy and safety in line with established research methodologies [18,23].

Emesis was induced in chicks using copper sulfate (50 mg/kg), as first described by Kinoshita *et al.* in 1996 with the approval of Meiji College of Pharmacy, Tokyo, Japan [29]. Subsequently, this method was replicated by Akita *et al.* in 1998 [22]. The experimental investigation revealed that the extracts of *C. gharaf* and *C. myxa* bark, leaves and fruits have prospective antiemetic effect. On the basis of investigation, it is found that extracts have anti-emetic potential when compared to reference standards i.e., chlorpromazine and domperidone. Chlorpromazine which is thus far reported to have an antiemetic effect via fastening the gastrointestinal tract movement [22] was observed to be lesser antiemetic than 150 mg/kg dose of the extracts of *Cordia gharaf* bark, leaves and *Cordia myxa*fruit shows a significant percentage of emesis inhibition with 90.55,72.70, and 83.72 % respectively. The phytoconstituents which exhibit antiemetic potential may be alkaloids, flavonoids and triterpenoids which is likewise endorsed through phytochemical study. Copper sulphate induced retching model is pondered to be commensurable with acute emesis and henceforth, aids as a suitable model for assessing the involvement of the brain in the observed anti-emetic effects of the *C. gharaf*and *C. myxa* extracts [23]. Emesis induced through copper sulphate is supposed to be linked to the peripheral 5HT receptors. The potential antiemetic effect of *C. gharaf* bark, leaves and *C. myxa* fruit extract might be due to anti-dopaminergic action, i.e. reducing the effect of dopamine at the D_2 receptor within the chemoreceptor trigger zone, that is how limiting emetic response to the medullary vomiting center and inhibition of serotoin interacting with 5-HT_4 receptor in addition to 5-HT_3 and stated as serotonin antagonist [24]. The search for new antiemetic agents from natural origin is based on bioactive compounds that emphasize on mechanism-based approaches. The phytochemical properties of the ethanolic extract from plants like *C. gharaf* and *C. myxa* have been investigated to understand their

potential antiemetic effects. While quantitative analysis may not have been conducted, qualitative observations and existing literature support a link between the phytochemicals present in these extracts and their ability to alleviate emesis. The presence of flavonoids and alkaloids in the extracts is highlighted in the phytochemical study. Flavonoids and alkaloids are known for their diverse biological activities, including their potential role in food value enhancement and medicine [25]. Specifically, flavonoids have been identified as having 5-HT3, 5-HT4, and NK1 receptor antagonist activity. These receptors are involved in the regulation of nausea and vomiting. By antagonizing these receptors, flavonoids may help alleviate symptoms of emesis [26]. Similarly, alkaloids present in the extract are noted for their anticholinergic action. Alkaloids often induce this effect by acting on M3 and M5 receptors, which are involved in cholinergic signaling pathways associated with emesis. By inhibiting cholinergic action within vestibular input to the vestibular nuclei and brainstem pathways, alkaloids may exert antiemetic effects [27, 28]. Existing literature supports the antiemetic potential of flavonoids and alkaloids. Previous studies have reported on the ability of these phytochemicals to alleviate symptoms of nausea and vomiting. This further strengthens the hypothesis that the flavonoids and alkaloids present in the ethanolic extracts from *C. gharaf* and *C. myxa* may contribute to their antiemetic activity[29, 30].

Limitations of the Study

The study has several limitations that need to be addressed. Firstly, the lack of quantitative analysis of the phytochemical composition of the ethanolic extracts from *C. gharaf* and *C. myxa* is a notable limitation. While qualitative observations suggest the presence of flavonoids and alkaloids, the absence of quantitative data makes it challenging to precisely assess the concentration and potency of these bioactive compounds. Without quantitative analysis, it is difficult to establish a direct correlation between the phytochemical composition of the extracts and their antiemetic effects.

Furthermore, relying solely on qualitative observations and existing literature to infer the potential antiemetic properties of the extracts has its limitations. While previous studies have reported on the antiemetic potential of flavonoids and alkaloids, extrapolating these findings to the specific extracts under investigation requires caution. The bioactivity of phytochemicals can vary based on factors such as extraction methods, plant species, and environmental conditions. Therefore, the applicability of findings from existing literature to the current study's extracts may not be straightforward.

Moreover, the mechanisms underlying the antiemetic effects of the extracts remain unclear. While the presence of flavonoids and alkaloids suggests possible pathways through which the extracts may exert antiemetic activity, further research is needed to elucidate these mechanisms. Understanding the specific molecular targets and signaling pathways involved in the antiemetic effects of the extracts would provide valuable insights into their pharmacological properties.

In light of these limitations, future studies should aim to address these gaps in knowledge. Quantitative analysis of the phytochemical composition of the extracts would provide a more comprehensive understanding of their bioactive constituents and their concentrations. Additionally, mechanistic studies are needed to elucidate the specific pathways through which the extracts exert their antiemetic effects. By addressing these limitations, future research can provide a more robust assessment of the potential of these extracts as natural antiemetic agents.

Future Avenues

Future research on the antiemetic potential of ethanolic extracts from *C. gharaf* and *C. myxa* should focus on several key areas to enhance our understanding and clinical applicability. Quantitative analysis of the extracts' phytochemical composition is essential to determine their potency and efficacy. Mechanistic studies are needed to elucidate the molecular pathways through which the extracts exert their antiemetic effects, particularly focusing on interactions with specific receptors such as 5-HT3, 5-HT4, NK1, and

cholinergic receptors. In vivo studies using animal models will provide valuable insights into pharmacokinetics, safety profiles, and dose-response relationships, informing potential clinical use. Subsequent clinical trials are necessary to validate the extracts' efficacy and safety in humans, comparing them with standard antiemetic medications. Optimization of extract formulations may enhance bioavailability and stability, while safety assessments are crucial to identify potential adverse effects. Through these research avenues, we can advance the development of ethanolic extracts from *C. gharaf* and *C. myxa* as safe and effective natural antiemetic agents.

Conclusion

The current study shows the antiemetic potential of the extracts of *C. gharaf* and *C. myxa*. The extract can be used for vomiting induced by gastroenteritis, anxiety, pregnancy and chemotherapy. Additional, research need to be done to authenticate the mechanism of action and also the phytoconstituents like flavonoids and alkaloids which are responsible for antiemetic action need to be isolated to validate their antiemetic potential along with toxicological studies so that we can produce potent natural antiemetics with less side effects.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

All animal protocols were performed as per guidelines of Animal Care and Use, National Institute of Health.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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