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Research Article

To An In – Vitro Evaluation of The Anthelmintic Activity of Piper Betle on The Pheritima Posthuma Model

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ABSTRACT

The leaves of piper leaves betle locally known as paan have long been in use in the Indian Indegenous system of medicine of the relief of pain however the underlying molecular mechanism of this effect the have not immunomodulatory effwcts of an ethanolic extract of the leaves of p. betle were demonstrated in a complete freund adjuvant induced model of arthritis in rats with dexamethasone as the positive control. At non –toxic concentration of PB a dose dependant decrease in extracellular production of nitric oxide in murine peritoneal microphages was measured by the grieas assay and corroborated by flow cytometry using the nitric oxide soecific probe 4,5 – diaminofluorescein -2 diacetate . the decreased generation of reactive nitrogen species was mediated by PB progressively down- regulating transcription of inducible nitric oxide synthase in macrophage , and concomintly causing a dose dependant decrease in macrophage in the expression of synthase in macrophage and concomitantly causing a dose – depedant decrease in the ability of PB to down – regulate T- helper 1 pro – inflamentary and anti-arthritis activity of PB is attributed to its bability to down regulate the generation of reactive nitrogen species this meriting further bpharmacological investigation.

INTRODUCTION

The research of new medicinal characteristics of diverse plant species has inspired the attention of the scientists towards the biologically active chemicals since the last couple of decades. This is because the bioactive chemicals are low- or non-

toxic and have strong pharmacological activity[1]. The widespread belief that herbal remedies are safer than synthetic medications with serious side effects and the resistance brought on by the careless use of synthetic medications are the primary causes of the growing interest in plant-derived medications[2]. Even though there are

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several antibacterial drugs accessible in medicine, the improper use and indiscriminate prescription of commercially available antibiotics have led to the development of multidrug resistance in human pathogenic microbes in recent years[3]. From the standpoint of underdeveloped nations today, synthetic medications are not only costly and unable to treat illnesses, but they can also have deadly side effects[4]. As a result, given the rise in the prevalence of newly discovered and reemerging pathogenic diseases, it is imperative to investigate novel antibacterial components with a variety of chemical structures and unique modes of action[5]. to take the place of those that are no longer effective. Additionally, we are aware that higher and aromatic plants have been used in traditional and folklore medicine to prolong the shelf life of goods that exhibit inhibition against yeasts and bacteria[6]. studies have reported that many herbs possess varying degree of antimicrobial activities. Therefore, the natural medicinal plants may be a potent source of new antibacterial agents. A portion of the body infested with parasitic worms such as roundworms (nematodes), tapeworms (cestodes), or flukes (trematodes) is known as helminthiasis[7]. Even though the worms live in the digestive system, they occasionally burrow into the liver and other organs. Anthelmintic medications either eradicate or kill helminthes, or worms, that are causing the infestation. Because of their strong anthelmintic characteristics, medicinal herbs have been studied for scientific advancement worldwide since ancient times[8]. Antihelmintic medications either eradicate or kill helminthes (worm infestations). Because of their strong anthelmintic effects, plants have been studied for their therapeutic qualities for the sake of science worldwide since ancient times[9]. Certain broad spectrum anthelmintics, such as albendazole and piperazine citrate, work effectively against both nematodes and parasitic flatworms. But most medications have a restricted

range of effects (like praziquantel), since resistance can emerge quickly[10]. There could be toxicity issues (Akhtar et al., 2000). Finding novel therapeutic plants with broad spectrum anthelmintic action and low toxicity is therefore imperative[11]. The primary sources of reactive oxygen species (ROS) in the forms of superoxide anions, hydroxyl radicals, and hydrogen peroxide are auto- oxidation of lipids and reactive nitrogen species (RNS). The oxidation or nitration processes of lipids, proteins, nucleic acids, and DNAs are likely to be harmed by the production of these extra ROS and RNS by ultraviolet (UV) radiation, smoking, and drug metabolisms. Conversely, these reactive oxygen species are linked to a number of degenerative illnesses, such as cancer, aging, arteriosclerosis, and rheumatism, and they can induce inflammation or lesions on different organs[12]. Naturally occurring phenolic compounds are well known for their capacity to donate hydrogen, chelate metals, reduce, and—most remarkably—capture free radicals and halt chain reactions. According to a report, the hydroxyl groups found in phenolic compounds may play a crucial role in scavenging free radicals and may directly contribute to the antioxidant activity. Plants with high polyphenol content have therefore gained more recognition as natural antioxidants across the globe[13]. The Piperaceae family of vines includes *P. betel* Linn. (Betel leaf), which is well known as paan in Bangladesh. It is mostly found in tropical and subtropical regions of the world[14]. *P. betel* leaves have several types of properties, including those that are respiratory depressive, cardi tonic, antitumor, antiulcer, antiplatelet aggregation, antifertility, and antitumor. In addition, it has aphrodisiac, tonic, anthelmintic, stomachic, and carminative properties. According to a number of reports, this plant's leaf has a wide range of advantageous bioactivities, and its extract has a lot of promise for application in the creation of commercial



goods[15]. According to numerous reports, this plant's leaf has a wide range of advantageous bioactivities, and its extract has a lot of potential for application in the creation of commercial goods. This served as the foundation for choosing this plant, especially the leaves. Therefore, using an in vitro study paradigm, the evaluation study seeks to establish the total phenolic components of *P. betel* leaves as well as their antibacterial and anthelmintic activities, all of which may be useful in the development of new, innovative medications[16].

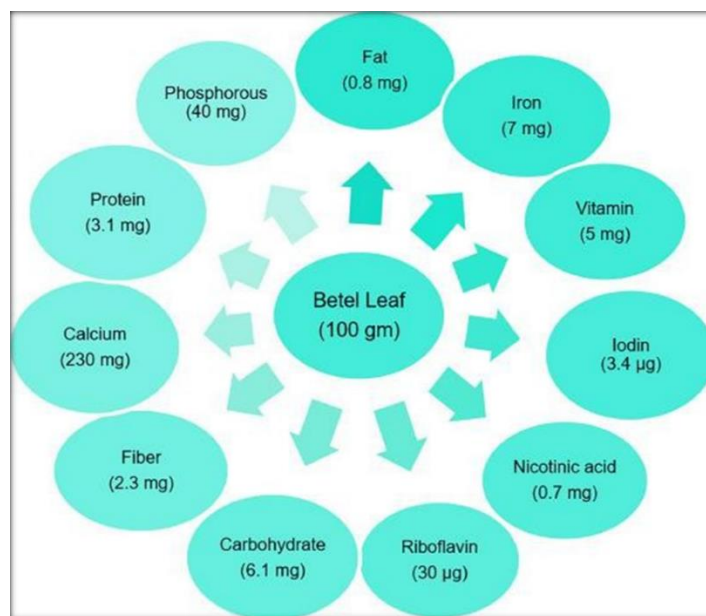


Table no 1[17].

Although there are many antibacterial agents available in the field of medicine, in recent years multidrug resistance has been developed in human

pathogenic microorganisms due to indiscriminate prescription and malpractice of commercially available antibiotics[18].

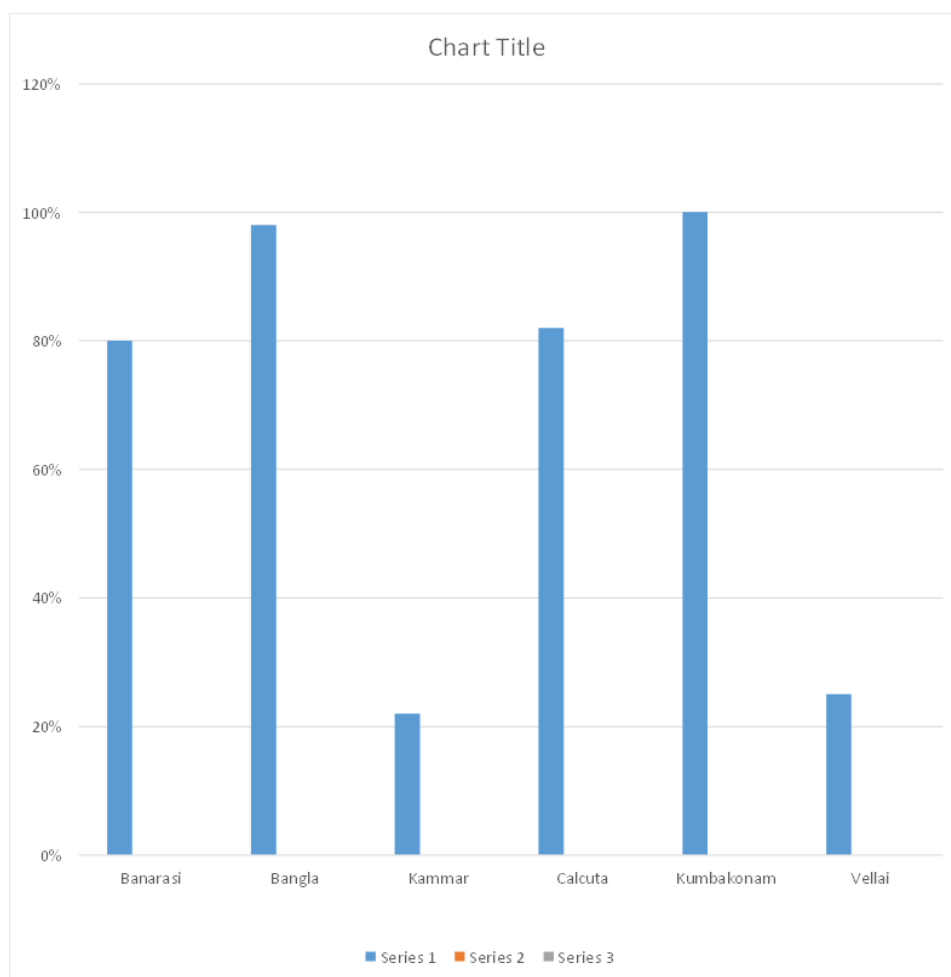


Table No 2[19].

Table No 3

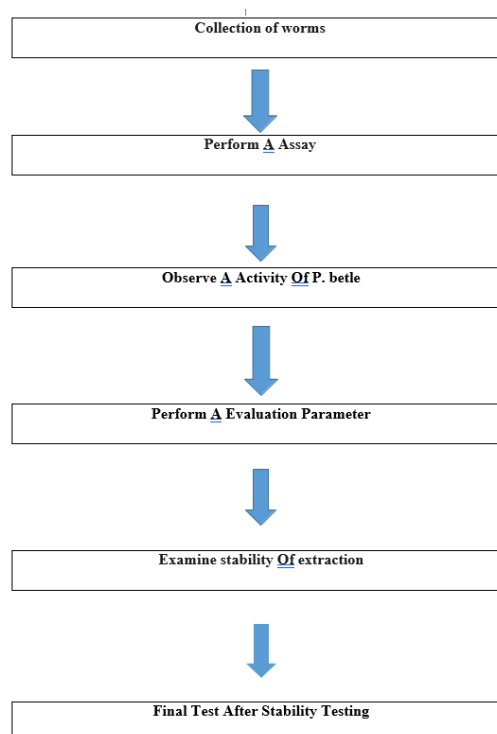
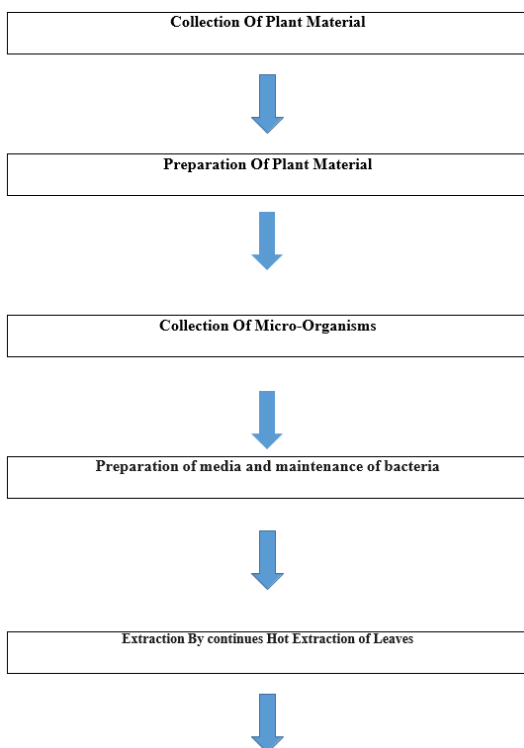
Components	Percentage Of Components
Chavibetol	53.1
Caryophyllene	3.71
chavibetol acetate	15.5
Allypyrocatechol Diacetate	0.17
Camphene	0.48
f-pinene	0.21
Eugenol	0.32
a-pinene	0.21
1,8-cineol	6.04

The Piper betle. (Piperaceae) leaves locally known as paan, It is used in Indian medicine system due to it's antioxidant and anthelmintic properties. Fresh leaves used as a post meal mouth freshener. It is cultivated in India, sri Lanka, Malaysia etc. It is evergreen plant. It is used as a antiseptic, it apply

on wound for healing purpose. Leaves are reach source of many nutrients like water, energy, Protein, fats, fiber, calcium, iron etc. Chief constituent of leaves is the volatile oil. It is used as tonic for brain, heart and liver. It promotes healthy teeth and skin. It is used as anthelmintic. It reduces

the cough. It gives analgesic and cooling properties. Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, ringworm, swelling of gum, rheumatism, abrasion, cuts and injuries etc as folk medicine while the root is known for its female contraceptive effects. The leaves are very nutritive and contain substantial amount of vitamins and minerals. The leaves also contain the enzymes like diastase and catalase besides a significant amount of all the essential amino acids except lysine, histidine and arginine, which are found only in traces. Anthelmintic drugs are used to treat infection with parasitic worms. It is important that anthelmintics are selectively toxic to the parasite and not the host. The mode of action of Albendazole is to cause paralysis of worms and expel them in the faeces[24].

PLANT OF WORK



BIOLOGICAL SOURCE

The betle (piper betle) is a vine belonging to the piperaceae family. which includes both pepper and kava .betel leaf is mostly chewed in asia , and elsewhere in the world by some Asian emigrants, as betel quid or in paan, with areca nut often with added tobacco.





Uses:

1. Antibacterial
2. Anticancer
3. Anti-diabetic
4. Anti-allergic
5. wound healing
6. Anti-inflammatory
7. Gastro protective
8. Anti-hypercholesterolemic [25]

MATERIAL AND METHODS

Collection and preparation of plant material

The leaves of *P. betel* were collected from Shirdi, Maharashtra in October 2024. The sun dried powdered leaves (500 mg) of *P. betel* was macerated in 2.5 L of 99.8% methanol (Merck KGaA, Darmstadt, Germany). After 15 days the solution was filtered using filter cloth and Whatman filter paper No. 1. The resulting filtrates were then evaporated in water bath maintained at 45°C to dryness and thus a blackish-green semisolid mass of the extract was obtained (yield 25 g).

Reagents and chemicals

All the solutions, reagents used in this study were of analytical grade.

Evaluation of the anthelmintic activity Collection of worms

The earthworms belonging to species *Pheritima posthuma* (Annelida), about 3-5 cm in length and 0.1- 0.2 cm in width weighing about 0.8-3.04 g, were collected from the moist soil

of Shirdi , Maharashtra, Sai Krupa Nersery. INDIA.

Reference Drug

For the evaluation of anthelmintic activity of *Piper betel*, the methanolic extract of leaves of the plant was tested in various doses in each group. Distilled water was used as control. Albendazole was used as the standard drug for comparative study with methanolic extract.

Anthelmintic assay

The anthelmintic assay was carried out as per the method of Adate et al. with minor modifications. Here the anthelmintic activity was assessed using earthworms because of their anatomical and physiological resemblance with that of the intestinal roundworm parasites of human being . They are widely used as effective tools for anthelmintic study because of their easy availability[20,21] . All of the worms were cleaned and all excrement was removed using regular saline water. Ten milliliters of distilled water were used to weigh the extracts and dissolve them in order to achieve concentrations of 10, 20, 40, 60, and 80 mg/ml. In a petri dish, earthworms were separated into seven groups, each with five worms. After applying the extract to the Petri dishes, the paralysis and death times were ascertained. Periods of paralysis occurred when there was no discernible movement of the worms, with the exception of when they were jolted violently. After determining that the worms did not move when shaken violently or when submerged in heated water (50°C), their body colors gradually faded and the time of death was recorded[22].

Determination of total phenolic content

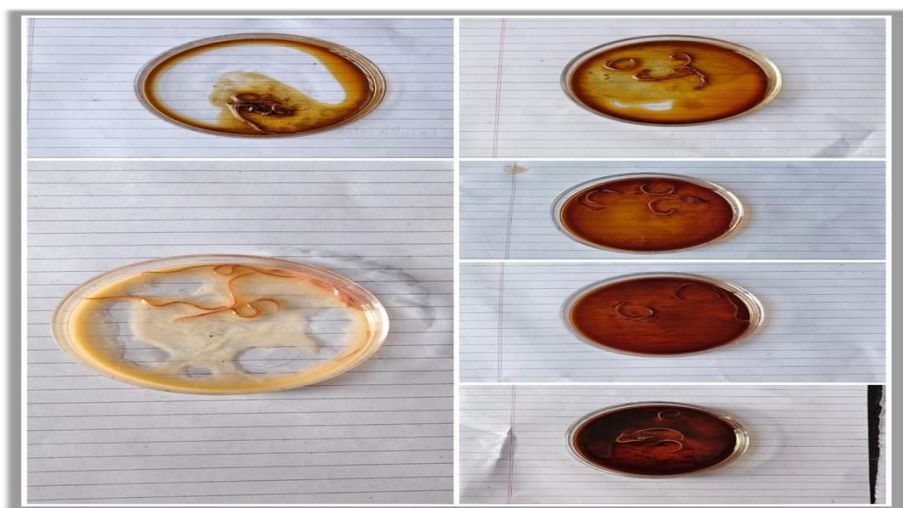
Total phenolic contents were determined by the Folin-ciocalteau method using Gallic acid as standard. The extract samples (0.5 ml of different dilutions) were mixed with Folin-ciocalteu reagent (2.5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na₂CO₃ (2 ml, 7.5 % w/v) was then added. The mixture was incubated for 20

minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer. The total phenolic content of the samples were measured using the standard curve prepared from Gallic acid solution with different concentrations (6.25, 12.5, 25, 50, and 100 $\mu\text{g/ml}$). The phenolic contents of the sample were expressed as mg of GAE (Gallic acid equivalent) / g of the extracts[23].

Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD) and were analyzed by One-way analysis of variance (ANOVA) (SPSS for windows, version 18.0, IBM corporation, NY, USA). The values were considered significantly different at $p < 0.05$.





CONCLUSION

Anthelmintic activity of *P. betel* extract

The crude methanolic extract of *P. betel* leaves was used to evaluate the anthelmintic activity and the activity of the methanolic extract was compared to that of standard drug albendazole. crude methanolic extract of leaves of *P. betel* revealed significant anthelmintic activity at the concentration of 10, 20, 40, 60 and 80 mg/ml in a dose dependent manner. It was also seen that at the concentration of 80 mg/ml the extract

demonstrated shortest time of paralysis and death time. At the concentration of 80 mg/mL, the methanolic extract caused paralysis of *Pheretima posthuma* at 4.16 ± 0.60 min and death at 5.16 ± 0.72 min, while Albendazole (positive control) caused paralysis and death at 19.33 ± 0.71 min and 51.00 ± 0.23 min, respectively at 10 mg/mL. From the study, it was also clear that the time for paralysis and death decreases as the increasing of concentrations of the extract. Therefore, the results demonstrate that methanolic extract of *P. betel* leaves possess wormicidal activity and thus may be used as an anthelmintic.

Table No 4

Group	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Distilled water)	-	-	-
Standard (Albendazole)	10	19.33±0.71	51.00±0.23
Methanolic extract	20	2.05±0.60	2.35±0.88
	40	1.27±0.29	1.59±0.33
	60	0.59±0.17	1.30±0.17
	80	0.38±0.44	1.00±0.60
	100	0.20±0.60	0.50±0.72

Determination of total phenolic contents of *P. betel* extract

shows the total phenolic contents of methanolic extracts of *P. betel* leaves. Total phenolic compounds were reported as gallic acid

equivalents by reference to a standard curve ($y=0.002x+0.107$; $R^2 = 0.889$). The results showed that the total phenol contents of methanolic extract was found 124.42 ± 0.14 mg of GAE/ g. of extract. The results of total phenolic contents suggest that the plant may possess good antioxidant activity.

Table No 5

Extract	Sl. No.	Absorbance of the sample	Average Absorbance	Total Phenolic Content (mg of GAE / gm.) of Extracts
Methanolic Extract	1	0.605	0.605 ± 0.0006	124.42 ± 0.14
	2	0.604		
	3	0.605		

DISCUSSION

Since decades, plants have proved to be a vital source of drug and many plants have been screened whether they contain compounds with therapeutic activity or not [26]. Therefore, it is very essential to evaluate the antibacterial activity of *P. betel*. The bacterial strains used in the current study were selected because of their clinical importance as they develop resistance against different antibiotics with their frequent uses. In the present study it was found that the mean zone of inhibition produced by the commercial antibiotic azithromycin was larger than that produced by methanolic extract of *P. betel* leaves. This fact may be explained by the postulate that the crude form of plant extract contains a lower concentration of bioactive compounds [27]. While screening medicinal plants for antibacterial activity, it is

generally expected that a greater number of compounds would be active against Gram positive rather than Gram negative bacteria [28]. However, in the present study it was found that the extract of *P. betel* was effective against both gram positive and gram negative bacteria which suggest that the plant extract may possess broad spectrum of antibiotic compounds or simply general metabolic toxin [29]. In a research conducted by Balaji et al. [30] using aqueous and ethanolic extract of leaves of *P. betel* indicated that the ethanolic extract of this plant showed better antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli* and moderate activity against *M. luteus* and *P. aeruginosa*. These results are quite similar to that of our present study although the sample preparation and some organisms were different. This may be described by the fact that the secondary metabolites responsible for

demonstrating antibacterial activity are greatly dependent on solvent system and collection process of metabolites from the plant sources [31]. Moreover, the geographical area and environment also affects the chemical composition of the plants and leads to the variation in activity [32]. Again, it was reported by several studies that several phytochemicals like terpenoids, flavonoids, tannins, alkaloids, steroids and some phenolic compounds are responsible for the antibacterial activity of the plant extract [33]. A study conducted by Al-Adhroey et al [34]. showed that the methanolic extract of the *P. betel* leaves contains certain phytochemicals like alkaloids, terpenes, anthraquinones, flavonoids, tannins, saponins and steroids. Whatever the mechanism, it is clear that some of these phytoconstituents of the plant extract may be responsible for the antibacterial activity. We know that parasitic helminthes affect human being and animals causing a chronic and debilitating disease which ultimately leads to death. Again, our traditional medicines hold a great promise as a great source of easily available effective anthelmintic agents to the people especially in developing countries. Many plants have reported to possess anthelmintic activity *in vitro* and *in vivo* [35]. In the current study observations were made for the time taken to paralysis and death of individual worms against the plant extract and the standard drug that is albendazole. It causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites and depletes their glycogen stores and ultimately leads to death [36]. The present study also revealed that leaves of *P. betel* showed potent anthelmintic activity. This may be described by the fact that several compounds like alkaloid,

polyphenol, flavonoid and terpene etc. may be responsible for the anthelmintic activity of the plant [37]. Several studies have confirmed that the leaves of *P. betel* are abundant of various phytochemicals [38]. among which some may be responsible for the wormicidal activity. these compounds may act on the CNS of the parasites causing paralysis and death of worms or interfere with the energy generation in the helminthes by uncoupling the oxidative phosphorylation or they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death [39]. The present study also estimated the phenolic contents of methanolic extract of *P. betel* leaves. It was reported that *P. betel* is a powerful source of both phenolic compounds and also of other phenolic acids such as gallic acid, gentisic acid, catechin and epicatechin [40]. Studies have also showed that the different levels of antioxidant activities in plants may be due to not only differences in their phenolic contents, but also in their phenolic acid components [41]. Because of the hydroxyl groups in the phenolic compounds, they may directly contribute to the antioxidant activity and have a critical role in scavenging free radicals [42]. Again, recent studies have shown that fruit and vegetable phenols and polyphenols such as flavonoids prevent free radical damage and lipid peroxidation [43]. The high content of total phenolic components in the methanolic extract may have led to the better results found in the total antioxidant activity and free radical scavenging ability of the plant. In conclusion from the recorded data, it is demonstrated that the methanolic extract of leaves of *P. betel* has promising antibacterial and anthelmintic effect. The extract also contains very prominent amount of phenolic compounds which may be responsible for its potent antioxidant activity. As the current study confirmed that leaves of *P. betel* showed several biological activities, so taking into



consideration of all the findings it can be mentioned that *P. betel* leaves can contribute major role in drug research. The plant may be further explored for its phytochemical profile to recognize the active constituents accountable for its versatile activities[44]

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