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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *ACALYPHA COMMUNIS* MULL. ARG. AGAINST INTOXICATION OF THIOACETAMIDE AND RIFAMPICIN-INDUCED RATS

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Keywords:

Acalypha communis Thioacetamide, rifampicin, Biochemical parameters and histopathological studies

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ABSTRACT: Background: The present study aimed to evaluate the hepatoprotective activity of aqueous extract of *Acalypha communis* in Wistar rats. **Methods:** The hepatoprotective activity was studied by Thioacetamide (TAA) (100 mg/kg b.wt.i.p) and Rifampicin (RMP) (100 mg/kg, po) induced models. Acute toxicity studies and preliminary phytochemical screening were also studied to evaluate the toxicity. **Results:** No toxicity profile was observed in rats after oral administration of the ethanolic leaf extract at 5mg/kg body weight. The different doses of 200 mg/kg and 400 mg/kg were administered with the extract of *Acalypha communis;* there was significant ($P < 0.001$) reduction in Biochemical parameters with respect to control. Phytochemical screening of the plant extract revealed the presence of tannins, alkaloids, flavonoids, saponins, and terpenoids. **Conclusion:** It can be concluded that the hepatoprotective activity elucidated by *Acalypha communis* could be mainly due to the presence of a high-value class of compound like the phenolic group as the major content in the plant.

INTRODUCTION: India has a rich culture of medicinal herbs and spices, which includes more than 2000 species, and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines, but only very few have been studied chemically and pharmacologically for their potential medicinal value [1, 2]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their Hepatoprotective activity. The plant *Acalypha communis* is a synonym of *Ricinocarpus communis* (Müll. Arg.), belonging to the family of Euphorbiaceae.

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It includes herbs, shrubs, and small trees, Shrubs or suffrutex frequently with resinous bright droplets on leaves and inflorescences; indumentum of simple or glandular hairs. Inflorescences spicate, usually unisexual [3].

MATERIALS AND METHODS:

Collection, Identification and Authentification of the Plants: The leaves of *Acalypha communis* Müll. Arg., were collected from the Malappuram district, Kerala, India, during the month of October 2019. The plant materials were identified and authenticated by Dr. Pradeep Botanist (CUPA No-BOT-11/08/2019AC) Calicut University, Kozhikode. Voucher specimens were kept in our laboratory for future reference.

Preparation of Extracts: The granulated dried leaves of *Acalypha communis* (500g) were packed in a Soxhlet apparatus and subjected to continuous hot percolation for 8 hrs using 450 ml of ethanol

(95% v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 75g, 15 % w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

Preliminary Phytochemical Screening: The extract was screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests [4, 5].

Procurement of Experimental Animals: Animals were selected as per the OECD guidelines. Healthy young and nulliporous, nonpregnant Sprague Dawleys female Rats weighing from 160-180 mg of 8–12 weeks old were selected because the literature survey of LD_{50} test shows that usually there is little difference in sensitivity between sexes.

Still, females were generally found slightly more sensitive when procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with a standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages.

The animals were kept under the alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No- P.Col/02/1868/26/09/2019/IAEC/JSPC) after scrutinization. The animals received the drug treatments by oral route.

Observations: Animals were observed individually for 48 hours after dosing at the first 30 minutes, periodically, and during the first 24 hrs, with special attention given during the first 4 hrs and daily after that, for a total of 14 days. Additional observations were also made if the animals displayed toxicity signs.

Changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns were included. Observations were also made and checked for tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma. Results were tabulated in **Table 2.**

Experimental Procedure [6, 7]: The rats were divided into five groups, each containing 6 rats.

Group I: Control rats received distilled water 5ml/kg/day *p.o.* for 28 days.

Group II: Rats treated with TAA (100 mg/kg b.wt.i.p) once daily for 28 days.

Group III: Rats treated with Silymarin (100 mg/kg) + TAA (100 mg/kg b.wt.i.p) once daily for 28 days.

Group IV: Rats treated with AC (200 mg/ kg, i.p.) + TAA (100 mg/kg b.wt.i.p) once daily for 28 days.

Group V: Rats treated with AC (400 mg/ kg, i.p.) + TAA (100 mg/kg b.wt.i.p) once daily for 28 days.

Experimental Procedure [8, 9]: The rats were divided into five groups, each containing 6 rats.

Group I: Control rats: Control rats received distilled water 5ml/kg/day *p.o.* for 28 days

Group II: Rats were treated with RMP (100 mg/kg, po) once daily for 28 days.

Group III: Rats treated with Silymarin (100 mg/kg) + RMP (100 mg/kg, po) once daily for 28 days.

Group IV: Rats treated with AC (200 mg/ kg, i.p.) + RMP (100 mg/kg, po) once daily for 28 days.

Group V: Rats treated with AC (400 mg/ kg, i.p.) + RMP (100 mg/kg, po) once daily for 28 days.

Statistical Analysis: The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way ANOVA followed by Dunnets Test to determine the significance level. Data were considered statistically significant at a minimum level of $p < 0.05$.

RESULTS:

Preliminary Phytochemical Screening: The preliminary phytochemical analysis of fractions of *Acalypha communis* shows the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin, and carbohydrate.

TABLE 1: ACUTE TOXICITY STUDY OF ETHANOLIC EXTRACTS OF LEAVES OF *ACALYPHA COMMUNIS IS* BASED ON OECD GUIDELINES 423

S. no.	Number of animals	Dose in mg/kg	Report
1	3	5mg/kg	No death
2	3	50mg/kg	No death
3	3	300mg/kg	No death
4	3	2000mg/kg	No death
5	3	5000mg/kg	No death

TABLE: 2 RESULTS OF GROSS BEHAVIORAL STUDIES IN RATS ON ADMINISTRATION OF *ACALYPHA COMMUNIS*

Observation	Effects								
Gross activity	Upto 3hrs	3 ½hrs	4 hrs	4 ½hrs	5hrs	5 ½hrs	6hrs	12hrs	24hrs
Respiration	+	+	+	+	+	+	+	+	+
Writhing	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-	-	-	-
Sense of touch and sound	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Diarrhoea	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-

TABLE 3: RESULTS OF THE EFFECTS OF BIOCHEMICAL MARKERS OF THIOACETAMIDE-INDUCED HEPATIC INJURY IN RATS

S. no.	Group/Drug	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
1	Group I- Normal control (NaCl 0.9% w/v)	5ml/kg	51.23 ± 2.31	60.12 ± 1 .24	27.17 ± 1.72	1.14 ± 0.13	8.74 ± 0.84
2	Group II- TAA	100mg/kg	162.8± 2.027#	183.6± 0.888#	149.5± 0.763 a ***	8.57± 0.0117#	3.30± 0.10
3	Group III- Silymarin+ TAA	100mg/kg + 100 mg/kg	58.50± 0.5627b ***	72.62± 0.7947**	93.90± 0.7765***	2.43± 0.069**	7.252± 0.0166***
4	Group IV – AC+ TAA	200mg/kg + 100 mg/kg	73.00± 1.461 b***	119.3± 0.9062*	124.1± 0.5498**	6.60± 0.0088*	5.385± 0.0133*
5	Group V – AC+ TAA	400mg/kg + 100 mg/kg	66.50± 0.7638 b ***	101.08± 0.8076**	116.1± 1.427***	3.39± 0.0036*	7.642± 0.0153**
6	Group VI– LC+ TAA	200mg/kg + 100mg/kg	80.00± 1.461 b***	127.3± 0.9062*	131.1± 0.5498**	6.93± 0.0088*	6.85± 0.0133*
7	Group VII – LC+ TAA	400mg/kg + 100mg/kg	69.50± 0.7638 b ***	116.08± 0.8076**	124.1± 1.427***	4.25± 0.0036*	7.032± 0.0153**

n=6; values were expressed Mean±S.E.M; Group II was compared to Group I. Groups III to V were compared to group II. *p < 0.01 vs. Thioacetamide group: significant; ** p < 0.001 vs. Thioacetamide group: highly significant Data were analyzed by One-way ANOVA followed by Dunnett's't' test.

TABLE 4: RESULTS OF THE EFFECTS OF BIOCHEMICAL MARKERS OF RIFAMPICIN-INDUCED HEPATIC INJURY IN RATS

S. no.	Group/Drug	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
1	Group I-Normal control (NaCl 0.9% w/v)	5ml/kg	51.23 ± 2.31	60.12 ± 1.24	27.17 ± 1.72	1.14 ± 0.13	8.74 ± 0.84
2	Group II- RMP	100 mg/kg	69.4 ± 6.4	112.4 ± 10.5a	118.6 ± 9.4a	7.55± 0.685	3.82± 0.31
3	Group III Silymarin+ RMP	100mg/kg + 100 mg/kg	36.5 ± 3.1**	69.21± 1.80***	30.65± 0.25***	3.21± 0.069**	7.61± 0.04***
4	Group IV – AC+RMP	200mg/kg + 100 mg/kg	38.5 ± 3.1*	84.8 ± 2.9*	65.8 ± 6.3**	4.60± 0.0088*	5.385± 0.0133*
5	Group V –	400mg/kg +	34.8 ± 2.9*	71.5 ± 6.2**	34.5 ±	3.39±	7.642±

	AC+RMP	100 mg/kg			6.9*	0.0036*	0.0153**
6	Group VI– LC+RMP	200mg/kg + 100 mg/kg	46.5 ± 3.1*	89.8 ± 2.9*	73.8 ± 6.3**	6.60± 0.0088*	4.385± 0.0133*
7	Group VII – LC+RMP	400mg/kg + 100 mg/kg	39.8 ± 2.9*	78.5 ± 6.2**	32.5 ± 6.9*	4.39± 0.0036*	6.642± 0.0153**

n=6; values were expressed Mean±S.E.M; ; Group II was compared to Group I. Groups III to V were compared to group II. *p < 0.01 vs. rifampicin group: significant; ** p < 0.001 vs. rifampicin group: highly significant Data were analyzed by One-way ANOVA followed by Dunnett's't' test.

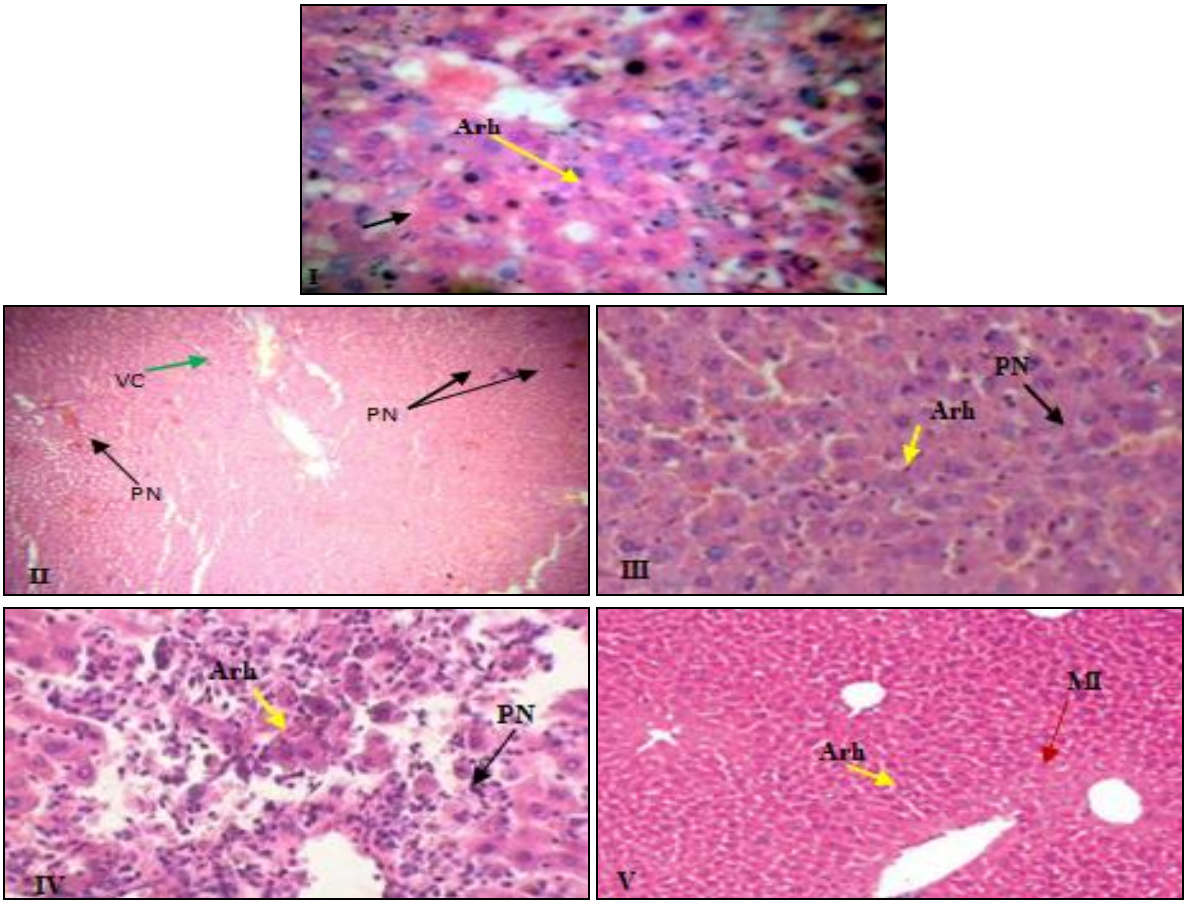
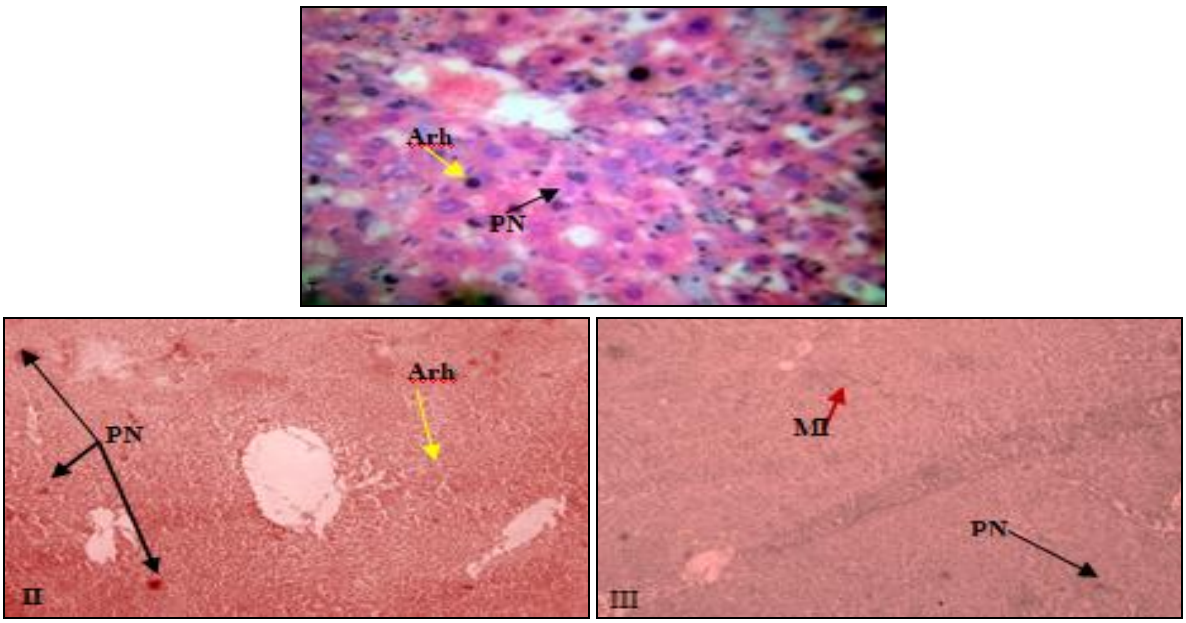


FIG. 1: HISTOPATHOLOGICAL STUDIES OF LIVER (THIOACETAMIDE INDUCED)



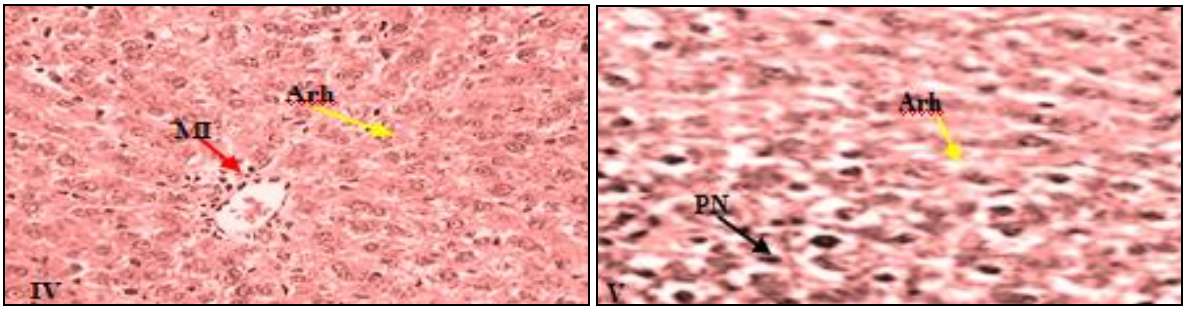


FIG. 2: HISTOPATHOLOGICAL STUDIES OF LIVER (RIFAMPICIN INDUCED)

DISCUSSION: The present study reveals the hepatoprotective activity of *Acalypha communis* against Thioacetamide and Rifampicin-induced hepatic damage in rats. Hepatotoxic drugs such as Thioacetamide and Rifampicin reduce liver functional capacity, which leads to an accumulation of waste products such as ammonia in the blood. The results show that *Acalypha communis* was effective in low and medium doses (200 mg/kg, p.o and 400 mg/kg, p.o).

One of the liver's major functions is detoxification of xenobiotics and toxins. TAA is a potent hepatotoxic agent metabolized by Cytochrome 450 enzyme present in the liver and is converted by oxidative chains to toxic substances called TAAS-oxide and TAAS-dioxide [10]. Now a day's Thioacetamide is a well-established tool used to induce hepatotoxicity in experimental animal models [11]. TAA is hepatotoxic and affects DNA, RNA, protein synthesis, and glutathione content, which, in turn, induces intra-hepatic metabolic changes [12, 13]. In this study, TAA administration to rats for 28 days has been observed to cause necrosis, increased mitosis at cells, apoptosis, abnormally mitosis, inflammation at portal space, and enlarged nucleus as assessed histopathologically. The mechanism of thioacetamide toxicity is due to the formation of thioacetamide-5-oxide, which is responsible for the change in cell permeability, increased intracellular concentration of Ca++, increase in nuclear volume, and enlargement of nucleoli, and also inhibits mitochondrial activity, which leads to cell death [14, 15]. Several researchers have suggested that part of hepatic cellular injury induced by TAA is mediated through oxidative stress caused by the action of cytokines through lipid Peroxidation [16, 17]. In our study, the rise in SGOT, SGPT, ALP and bilirubin levels induced by TAA administration was significantly reduced by AC pre-treatment, suggesting its hepatoprotective activity. The histopathological findings showed that treatment with AC extract recovered liver structure in TAA-induced liver cirrhosis in rats. Indeed, there was a remarkable reduction in the extent of fibrosis, probably due to reduced amount of stellate cells infiltration in rats treated with the plant extracts compared to the TAA group. TAA-treated rat liver showed fatty degeneration and necrosis. These effects were nearly normalized in the histo architecture of livers in the *AC*-treated rats, especially in the high dose (*AC* 400 mg/kg) group

Tuberculosis (TB) has been a leading health problem for many years and remains a major cause of death worldwide. Isoniazid (INH) and rifampicin (RMP), the most important first-line antitubercular drugs (ATD) have been used for the treatment of TB. However, a variety of adverse reactions have been reported. One of the well-known toxic effects is hepatotoxicity [18]. A meta-analysis of studies involving several antituberculosis drug regimens estimates the incidence of liver toxicity is 2-6% with co-administered isoniazid and rifampicin and 1.1% with rifampicin alone [19]. Xenobiotics, including antituberculosis drugs, undergo biotransformation in the liver catalyzed by microsomal enzyme systems. The mechanism of RMP-induced liver injury is not yet understood fully. Several studies have shown that RMP causes oxidative injury to the liver, its membrane, and organelles, leading to lipid peroxidation and depletion of the antioxidant glutathione (GSH) and the free radical scavenging enzymes [20]. Rifampicin inhibits both uptake and excretion of bilirubin in a dose-related manner, giving rise to elevated plasma levels of both conjugated and unconjugated bilirubin [21]. several reactive derivatives of drugs and oxidants are generated during the process of drug biotransformation. The reactive species generated can bind and/or react with cellular

components in the liver and cause liver injury resulting in impairment of liver functions. The reaction of reactive species with cellular antioxidants causes antioxidant depletion that may result in oxidative stress [22]. Rifampicin causes transient elevations in hepatic enzymes, usually within a few weeks of therapy in 10% to 15% of patients, with less than 1% of the patients demonstrating overt rifampicin-induced hepatotoxicity [23]. Cytochrome P450

mediates generation of reactive metabolites of drugs, and their covalent binding to hepatic macromolecules is the most accepted mechanism of RMP-induced hepatic injury [24, 25]. Daily administration of Rifampicin drugs for 28 days results in hepatic injury as confirmed by elevated serum diagnostic enzymes such as SGOT, SGPT ALP, and Total bilirubin levels. At the time of hepatic injury, these enzymes leak out from the liver into the blood circulation due to liver tissue damage. The histological profile of control animals revealed normal architecture with central veins and portal triad. Animals treated with rifampicin exhibited focal haemorrhage, inflammation, and bridging necrosis. Pre-treatment with *AC* reduced focal haemorrhage, inflammation, and bridging necrosis induced by rifampicin.

This study shows that hepatic injury induced by Thioacetamide and Rifampicin caused a significant rise in marker enzymes SGOT, SGPT, ALP, and total bilirubin. The serum enzymes like SGOT, SGPT, ALP, and total bilirubin of treated animals were significantly reduced ($p<0.01$) by 28 days pre-treatment of ethanolic extract of leaves of *Acalypha communis* at two dose levels 200 mg/kg and 400 mg/kg p.o, when compared with Thioacetamide and Rifampicin treated (group-II). From the result, it is clear that the drugs show dose-dependent activity. Histopathological observation also revealed that pre-treatment with *Acalypha communis* protected the animals from Thioacetamide and Rifampicin-induced liver damage. The results indicate that the leaves of *Acalypha communis* possess Hepatoprotective activity.

CONCLUSION: From the present work, we conclude that species of *A. communis* are highly potential in biological activity. The preliminary screening of the samples revealed the presence of a high-value class of compound-like phenolic group as the major content in the plants.

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