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Antioxidant Efficacy of Bark and Leaf of *Homonoia retusa* (Graham ex Wight) Müll. Arg.

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ABSTRACT

Homonoia retusa (Graham ex Wight) Müll. Arg.rheophytic shrub traditionally used against ulcers and renal calculi. Fresh plant materials were air-driedpowder blended into a fine powder and subjected to Soxhlet extraction. Preliminary screening of ethanolic extracts ofleaf and bark extracts showed the presence of a diversity of phytochemical constituents. Tannins and Phenols, Saponins, and Phytosterols were detected in both bark and leaf extracts. The antioxidant capacity DPPH free radical scavenging assay of the extracts was compared with ascorbic acid as a standard antioxidant. Leaf extract exhibited higher antioxidant activity (77.6%) than the bark extract (71.4%).Phosphomolybdenum (PM) assay showed the highest activity among bark extract (76.9%) and leaf extract (75.5%) standard ascorbic acid. It is evident to conduct further studies and investigate the lead compounds present in the plant evaluate its potentiality in vivo animal models and put forward an attempt to carry out trials on human beings.

Keywords: Antioxidant; *Homonoia retusa*; DPPH; Medicinal plants; Phytochemicals; Phytomedicine; PM assay;

I. INTRODUCTION

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenicity, anti-carcinogenicity, and antiaging, among others, originate from this property however, information on the relationship between antioxidant activity and phenolic content and composition of many food plants is not available (Velioglu, et al., 1998).The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower incidence and lower mortality rates of cancer in several human cohorts (Zheng, et al., 2001).

Herbal medicines have been widely used and now form an integral part of primary healthcare in many countries. They may constitute a reservoir of new antimicrobial substances to be discovered. Roots of *Homonoia riparia* Lour. is used as a laxative, diuretic and emetics. A decoction of the root is given for piles, stones in the bladder, chest pain, gonorrhea and syphilis. Powdered leaves and fruits are applied as poultices for skin diseases (Bapat & Mhapsekar, 2014). *H. retusa* is mostly seen along the banks of streams and rocky places. Root is laxative and diuretic. Decoction of the root is used in the treatment of piles, stones in the bladder, gonorrhea and syphilis. The root is used against ulcers and vesical calculi (Pattasseril, et al., 2015).

Homonoia retusa has been long used in traditional medicines to treat a broad range of illnesses, i.e., Malaria, bladder stone, urinary discharge, inflammation, ulcer, uterine disorders and blood disorders. Additionally, the plant's roots have emetic, anti-urolithiasis, diuretic, and laxative properties. The fruits, flowers and leaves are used to treat inflammation, cuts, antibacterial, antifungal, anticancer and dermatitis (Porika and Reddy, 2022).

II. MATERIALS AND METHODS

Collection and Preparation of plant material

Homonoia retusa (Graham ex Wight) Müll. Arg.is a riparian shrub located along the bank of rivers, it was collected from the bank of river Tungabhadra, Udagatti village (14°42'08.0" N 75°44'24.8" E), Ranebennur taluk, Haveri district, Karnataka. Fresh plant materials (twigs) were

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collected sustainably brought to the laboratory and washed with water. Further, the leaves were separated, and bark was peeled off from the fresh plant materials were air dried under shade to remove moisture. After drying then blended into fine powder. The powder was stored in airtight containers for further use. About 200 g of dried leaf and bark material was crudely powdered and subjected to extraction by a Soxhlet extractor. The extraction was done using ethanol as solvent. All the extracts were concentrated by rotary vacuum evaporator (Super Fit – 2.0 model) and the left-over solvent was evaporated to dryness using a water bath **(Figure1)**.

Preliminary Phytochemical analysis

The phytochemical analysis was carried out by using ethanolic extracts of leaf and bark. Qualitatively tested for different phytochemical constituents namely Carbohydrates, Alkaloids, Flavonoids, Coumarins, Anthocyanins, Emodin, Proteins and amino acids, Saponins, Cardiac-glycosides, Gums and Mucilage, Tannins and Phenols, Terpenoids, Diterpenes, Triterpenes and Phytosterols by following the standard procedures followed by standard procedures (Deepti, et al., 2012; Shaikh & Patil, 2020).

Antioxidant assay

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

Free radical scavenging effect of *Homonoia retusa* leaf and bark extracts was determined using the stable scavenger DPPH with slight modifications of the method described by Brand Williams, et al., (1995) briefly, the concentrations (100 µL to 500 µL) of extracts were prepared in ethanol. DPPH solution (0.004%) was prepared in ethanol and 1 ml of this solution was mixed with the same volume of methanol, ethanol, and aqueous leaf extracts and standard ascorbic acid solution separately. The mixture was incubated for 30 min in the dark at room temperature and the absorbance was measured at 517 nm. The degree of DPPH-purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

Blank = Control = Methanol

The scavenging activity against DPPH was calculated using the equation:

DPPH scavenging activity (%) = A_C –A_S /A_C × 100

Where,

Ac - is the absorbance of the control reaction (1 ml of ethanol with 1 ml of DPPH solution).

As - is the absorbance of the test sample.

The results were analyzed in triplicate. The IC_{50} value is the concentration of the sample required to inhibit 50% of the DPPH free radical.

Phosphomolybdenum (PM) assay

Total antioxidant activity was estimated by PM assay using the standard procedure of Prieto et al. 1999 and Puraniket al., 2017. Ethanolic extracts of leaf and bark of H. retusa in different concentrations ranging from 20 µL to 200 µL were added to each test tube individually containing 3 mL of distilled water and 1 mL of molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, they are kept in room temperature for 20-30 min. and the absorbance is measured at 695 nm. Ascorbic acid is used as a reference standard.

III. RESULTS

Homonoia retusa a small and rigid, rheophytic prostrate shrub that spreads up to 2 m. The stem is slightly brown and woody. The total yield of crude extracts from bark and leaf by using the. ethanolic extract of leaf 21.09 gm and bark 19.52 gm (out of each 200g taken) with the reference to the air-dried plant material **(Figure 1).**

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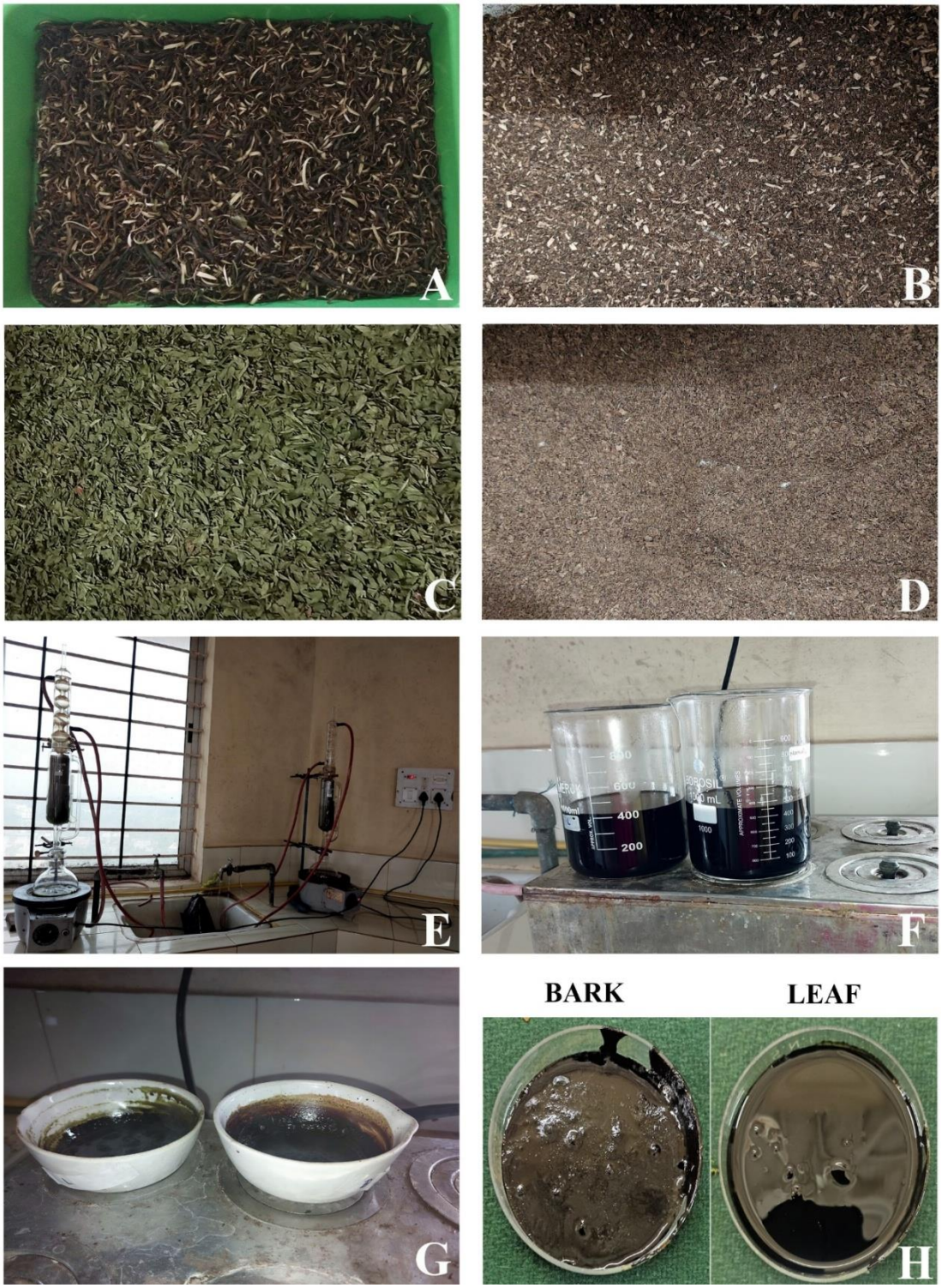

Figure 1. *Homonoia retusa* (Graham ex Wight) Müll. Arg. **A.** Bark (peeled) shade drying; **B.** Bark Powder; **C.** Leaf shade drying; **D.** Leaf Powder; **E.** Soxhlet extraction apparatus; **F. & G.** Evaporating the mother solvent from the extracts by water bath; **H**. Extracts.

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Preliminary screening of ethanolic extracts ofH. retusa leaf and bark extracts showed the presence of a diversity of phytochemical constituents. Tannins and Phenols, Saponins, and Phytosterols were detected in both bark and leaf extracts.

Table. 1: Preliminary phytochemical screening of ethanolic extracts of *Homonoia retusa* (Graham ex Wight) Müll. Arg.

Constituents	Tests	Leaf extracts	Bark extracts
Carbohydrates	Molisch's	+	+
	Benedict's	+	+
	Fehling's	+	+
Alkaloids	Dragendroff's	+	+
	Mayer's	-	-
	Hager's	-	-
	Wagner's	-	-
Flavonoids	Shinoda	+	+
Coumarins	Alkaline	+	+
Anthocyanins	HCl	-	-
Emodin	Emodin test	-	-
Proteins and amino acids	Xanthoproteic	+	+
Saponins	Froth formation	+	+
Cardiac-glycosides	Keller - Killani	-	-
	Baljet's	-	-
Gums and Mucilage	Alcohol	-	-
Tannins and Phenols	$FeCl_3$	+	+
	Gelatin	+	+
Terpenoids	Test - 1	-	-
Diterpenes	Test - 2	-	-
Triterpenes	Test - 3	-	-
Phytosterols	Salkowski's	+	+
	Hesse's response	+	+

+: Present; -: Absent

Antioxidant assay

DPPH radical scavenging activity

In the present study, different concentrations of leaf and bark ethanolic extracts of *Homonoia retusa* were subjected to a DPPH free radical scavenging assay (**Table 2**). The antioxidant capacity of the extracts was compared with ascorbic acid as a standard antioxidant. Leaf extract exhibited higher antioxidant activity of (77.6%) than the bark extract (71. 4%) **(Figure2).**

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Table 2. Determination of percentage of DPPH radicle scavenging activity of Ascorbic acid (Standard) Leaf Extract (LE) and Bark Extract (BE) (Sample).

Concentration in µg/ml (AA)	% of inhibition by Ascorbic acid (AA)	% DPPH radical scavenging activity in Leaf Extract (LE)	% DPPH radical scavenging activity in Bark Extract (BE)
20	55.36	36.04	32.56
40	59.63	43.56	41.59
60	61.71	49.23	46.20
80	64.02	54.70	53.02
100	69.33	59.58	57.36
120	74.96	62.35	60.00
140	78.66	66.89	61.94
160	82.63	69.63	64.89
180	83.52	74.98	68.47
200	91.32	77.57	71.39

Pattasseril, et al., 2015 studied phytochemical analysis of *H. retusa* extracts it showed that most of the secondary metabolite (Carbohydrates, Flavonoids, and Aanthocyanosides) groups have shown their presence in ethanolic and water extracts as compared to other solvent systems. Pattasseril, et al., 2017 investigated in vitro antioxidant activity of *H. retusa* by hydroxyl radical scavenging activity, superoxide free radical scavenging activity, DPPH assay, nitric oxide radical scavenging activity, total antioxidant activities were conducted using ethanol, chloroform, and ethyl acetate solvents. In the DPPH assay ethyl acetate extract resulted in a maximum of 96.18% followed by 94.51 % and 84.32 % in chloroform and ethanol extracts respectively.

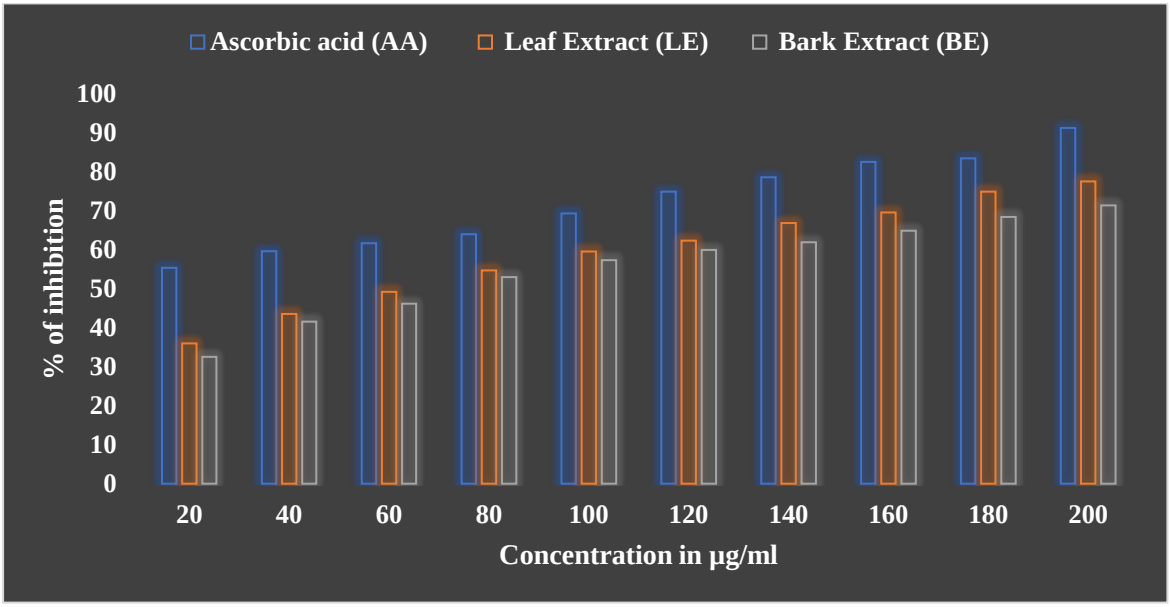

Figure 02.DPPH assay for *Homonoia retusa* leaf (LE) and bark (BE) ethanolic extracts and ascorbic acid (AA) as standard.

Phosphomolybdenum (PM) assay

In the present study, ethanol extract was subjected to PM assay along with standard ascorbic acid. Ethanol, extract showed the highest activity between leaf and bark extracts and standard ascorbic acid (Table 3 &Figure3). Out of them, bark extract (76.9 %) showed the highest activity and leaf extract (75.5 %) in ethanolic extract.

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Table 3. Determination of percentage of PM scavenging activity of Ascorbic acid (Standard) Leaf Extract (LE) and Bark Extract (BE) (Sample).

Concentration in µg/ml	% of inhibition by Ascorbic acid (AA)	PM scavenging activity in Leaf Extract (LE)	PM scavenging activity in Bark Extract (BE)
20	48.4	36	34.6
40	59.6	39.5	37.5
60	61.7	42.3	42.2
80	66	54.7	49.2
100	70.3	59.5	55.6
120	72.9	62.3	60
140	77.9	68.8	63.4
160	81.4	70.6	68.9
180	83	73.9	70.7
200	89.2	75.5	76.9

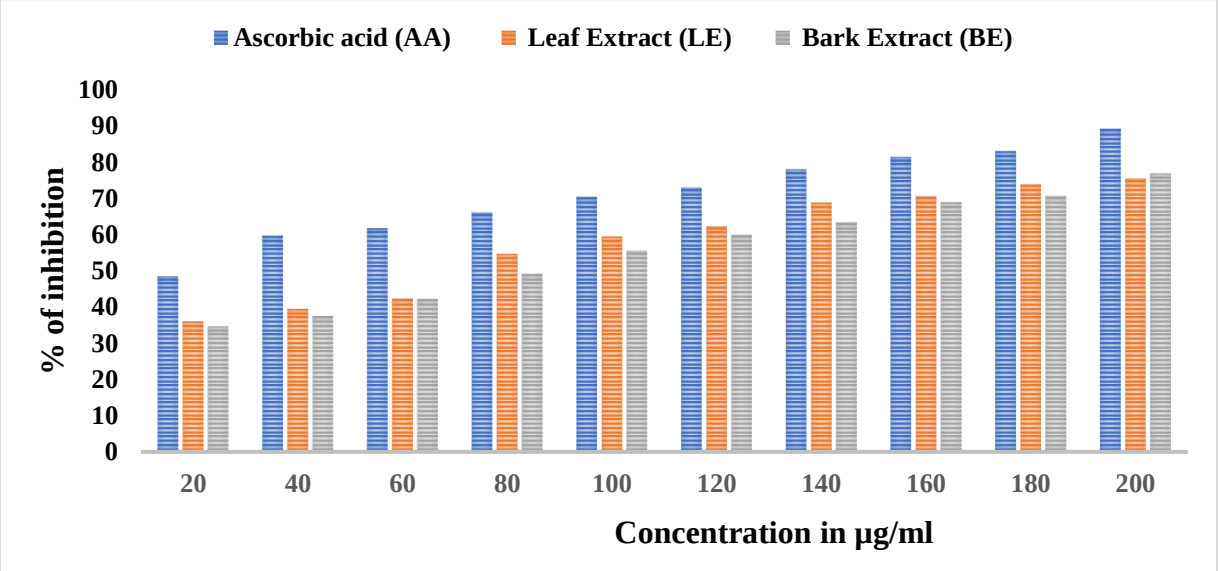

Figure 03.Phosphomolybdenum (PM) assay for *H. retusa* leaf (LE) and bark (BE) ethanolic extracts and ascorbic acid (AA) as standard.

IV. CONCLUSION

Homonoia retusa contains a wide varietyof secondary metabolites like Saponins, Tannins and Phenols, Phytosterols, and Carbohydrates. DPPH assay of ethanolic leaf extracts showed maximum activity compared to bark extract. In PM assay ethanolic bark extract shows the highest activity and leaf extract (75.5 %) and standard ascorbic acid. It gives scientific evidence to conduct further studies to investigate the lead compounds present in the plant evaluate its potentiality in vivo animal models and put forward an attempt to carry out trials on human beings.

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