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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF MEDICINAL PLANTS

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ABSTRACT

Objective: The present study was carried out to evaluate the antioxidant activity of different extracts of two medicinal plants.

Methods: *In-vitro* antioxidant activity of different extracts of *Hydrocotyle javanica* Thunb and *Peristrophe bicalyculata* (Retz.) Nees were determined by following methods such as Lipid peroxide assay (LPO), nitric oxide assay (NO), glutathione (GSH), catalase (CAT).

Results: Among eight different extracts, n-Hexane *H. javanica* extract and alcoholic *P. bicalyculata* extract showed more potent *in vitro* antioxidant activity in terms of LPO, NO, GSH, SOD, and CAT assay.

Conclusion: From the results, it can be concluded that n-Hexane *H. javanica* extract and alcoholic *P. bicalyculata* extract showed more potent *in vitro* antioxidant activity.

Keywords: *Hydrocotyle javanica*, *Peristrophe bicalyculata*, Lipid peroxide, Nitric oxide, Glutathione, Superoxide dismutase, Catalase assay.

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INTRODUCTION

Antioxidants are compounds that inhibit or delay oxidation of other molecules by inhibiting both initiation and propagation of oxidizing chain reactions [1]. Reactive oxygen species, such as superoxide anion (O_2^-) radicals, hydroxyl radicals (OH•), and hydrogen peroxide (H_2O_2), can cause oxidative damage to macromolecules, including DNA, proteins, lipids, and small cellular molecules [2]. Free radicals have been implicated in the pathology of many diseases, including cancer, atherosclerosis, diabetes, and neurodegenerative disorders, in addition to aging [3,4]. Medicinal plants contain a high amount of antioxidant. Herbal plants are traditionally used as medicine and it is considered to be harmless which is consumed or taken by many people without any prescription [5].

Hydrocotyle javanica (*H. javanica*) Thunb. is a naturally growing prostrate herb found throughout the Himalayas and Assam Hills, Nilgiri Hills, and Western Ghats of India at altitudes of 2000–8000 ft [6]. The plant belongs to the family Apiaceae (formerly known as Umbelliferae) and the subfamily is Hydrocotyloideae [7]. Conventionally, the fresh plant parts of *H. javanica* Thunb. are used as crushed and ingested to cure sore throats and lungs. Kurichiya tribes of Kerala (India) apply the juice of the whole plant on the chest to cure asthma and convulsions [8]. Pharmacologically, it had been proved that the methanolic fraction of the plant showed antibacterial activity against some human pathogenic bacteria which had been published in our earlier publication [9].

Peristrophe bicalyculata (*P. bicalyculata*) is up to 60–180 cm in height and found almost throughout India, Afghanistan, and Africa. It is commonly known as kali aghedi in Hindi and Kakajangha in Sanskrit. The herb is used for its antibacterial property (tuberculostatic), snake poison, in bone fracture and sprain. Leaf extract is used for fever, cold, and cough. Mucilage medicines are used for ear and eye treatments [10-16]. The chemical composition of the dried aerial parts of *P. bicalyculata* reveals that is comprised 14-methyltritriacont-14-en-15-ol and 35-hydroxy-nonatriacontanes [17]. The essential oil shows tuberculostatic activity *in vitro* against the growth of various strains of *Mycobacterium tuberculosis* [18].

METHODS

Collection and identification

Collection of two medicinal plants *H. javanica* and *P. bicalyculata* was carried out during the year 2017–2018 on the basis of its medicinal information given by various taxonomists in Pondicherry. Targeted collection based on chemotaxonomic relationships and ethnomedical information derived from traditional medicines. The identified plants were authenticated by Dr. N. Loganathan Specialist in Medicinal plants, Hereditary Physician, Pondicherry.

Preparation of extract

The collected plant materials were washed with distilled water to remove dirt and soil. The whole plants were further shade dried and then coarsely powdered. The coarse powder of two plants (500 g) was extracted with 3 L of different solvents such as petroleum ether (60–80°C), hexane (68.5–69.1°C), chloroform (55.5–61.5°C), alcohol 95%, and water by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in desiccator. The extract was vacuum dried and processed further for pharmacological evaluation [19-21].

Phytochemical screening

The different extracts of *H. javanica* and *P. bicalyculata* were subjected to various chemical tests for the identification of phytochemical constituents (glycosides, phytosterol, saponins, alkaloids, carbohydrates, flavonoids, tannins, protein, and amino acid) [22-24].

Evaluation of *in vitro* antioxidant activity of different extracts of *H. javanica* Thunb. and *P. bicalyculata* (Retz.)

Preparation of cell lysates

After the study period, the medium was aspirated and cells were washed with the ice-cold phosphate buffer saline, scraped, and were centrifuged at 5000 rpm for 5 min at 4°C. The cell pellets were resuspended in 2000 µl of lysis buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1% Triton X-100, 5 mM EDTA, 50 mM NaF, 100 µM Na3VO4, 1 mM PMSF, 10 µg/ml

leupeptin, and 10 μg/ml aprotinin) and incubated on ice for 30 min. The cell lysates were obtained by centrifugation at 12,000 rpm for 20 min at 4°C. Cell lysates obtained were stored at −20°C until use.

Lipid peroxide (LPO) assay

Lipid peroxidation was evaluated in cell lysates by measuring the malondialdehyde content according to the TBA test [25] with slight modification. 0.2 ml of the cell lysate was taken and to this 0.8 ml saline, 0.5 ml of BHT and 3.5 ml TBA reagent (0.8%) were added and incubated at 60°C. After cooling, the solution was centrifuged at 2000 rpm for 10 min. The absorbance of the supernatant was determined at 532 nm using spectrophotometer against the blank [25].

Nitric oxide (NO) assay

NO was assayed by taking 0.2 ml of medium followed by addition of 1.8 ml of saline and 0.4 ml of 35% sulfosalicylic acid for protein precipitation. The precipitate was removed by centrifugation at 4000 rpm for 10 min. To 1 ml of aliquot of supernatant, 2 ml Griess reagent (1 g of sulfanilamide dissolved in small volume of water, 2 ml of orthophosphoric acid, and 100 mg of naphthyl ethylenediamine were added and the final volume was made up to 100 ml with distilled water). The mixture was allowed to stand for 20 min under dark conditions. The color intensity of the chromogen was read at 540 nm. Standard calibration curve was plotted using sodium nitrite in the concentration range 200–1000 ng [26].

Reduced glutathione (GSH)

GSH content was estimated by following the method [27]. 0.25 ml of cell lysate was added to an equal volume of ice-cold 5% TCA. The precipitate was removed by centrifugation at 4000 rpm for 10 min. To 1 ml aliquot of supernatant, 0.25 ml of 0.2 M phosphate buffer (pH 8.0) and 0.5 ml of DTNB (0.6 mM in 0.2 M phosphate buffer, pH 8.0) were added and mixed well. The absorbance was read at 412 nm using spectrophotometer (UV, Shimadzu, Japan) [28].

Superoxide dismutase (SOD)

The sodium pyrophosphate buffer (0.025 M, pH 8.3) in a quantity of 0.3 ml was added to 0.05 ml of cell lysate. To this mixture, 0.025 ml and 0.075 ml of PMS (186 μM) and NBT (300 μM in buffer, pH 8.3) were added. The initiation of the reaction was commenced by the instillation of 0.075 ml of NADH. The mixture was then incubated at temperature of 30°C for a period of 90 seconds. 0.25 ml of glacial acetic acid was added to arrest the ongoing reaction. N-butanol (2 ml) was shaken vigorously along with the reaction mixture; later, the mixture was centrifuged at 4000 rpm for 1 min. The colorimetric analysis was carried out at 560 nm using spectrophotometer, with n-butanol (1.5 ml) serving as blank [29,30].

Catalase (CAT)

A small quantity of cell lysate (100 μl) or sucrose (0.32 M) was subjected to incubation with potassium phosphate buffer (2.25 ml) 65 mM at pH 7.8 for 30 min at 25°C. The initiation of the reaction was by the addition of H_2O_2 (7.5 mM; 650 μl). The absorbance change was measured for a period of 2–3 min at 240 nm (UV, Shimadzu, Japan) [27,30-32].

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screenings of various extracts of *H. javanica* and *P. bicalyculata* mainly revealed the presence of glycosides, phytosterol, saponins, alkaloids, carbohydrates, flavonoids, tannins, protein, and amino acid, as shown in Tables 1 and 2, respectively.

Evaluation of *in vitro* antioxidant activity of different extracts of *H. javanica* Thunb. and *P. bicalyculata* (Retz.)

Different extracts of *H. javanica* Thunb. and *P. bicalyculata* (Retz.) were evaluated for *in vitro* antioxidant activity using LPO assay, NO assay, GSH assay, SOD, and CAT. Among eight different extracts, n-Hexane *H. javanica* extract and alcoholic *P. bicalyculata* extract showed more potent *in vitro* antioxidant activity, as shown in Figures 1-5.

Table 1: Phytochemical screening of different extracts of *H. javanica*

S. No.	Phytoconstituents	Pet. ether	n-Hexane	Chloroform	Alcohol	Aqueous
1.	Alkaloids	−	−	+	+	+
2.	Carbohydrates	−	−	−	+	+
3.	Glycosides	−	+	−	−	−
4.	Phytosterols	+	+	−	+	+
5.	Saponins	−	−	−	−	+
6.	Fixed oils and fats	−	−	−	−	−
7.	Tannin and phenolic compounds	−	−	−	−	+
8.	Proteins and free amino acids	−	−	+	+	+
9.	Gums and mucilage	−	−	−	−	−
10.	Flavonoids	−	−	+	+	+
11.	Lignin	−	−	−	+	+
12.	Volatile oil	−	−	−	−	−

(+) Indicates presence, (−) indicates absence. *H. javanica: Hydrocotyle javanica*

Table 2: Phytochemical screening of different extracts of *P. bicalyculata*

S. No.	Phytoconstituents	Pet. ether	n-Hexane	Chloroform	Alcohol	Aqueous
1.	Alkaloids	−	−	+	+	+
2.	Carbohydrates	−	−	−	+	+
3.	Glycosides	−	+	−	−	−
4.	Phytosterols	+	+	−	+	+
5.	Saponins	−	−	−	+	+
6.	Fixed oils and fats	−	−	−	−	−
7.	Tannin and phenolic compounds	−	−	−	−	+
8.	Proteins and free amino acids	−	−	+	+	+
9.	Gums and mucilage	−	−	−	−	+
10.	Flavonoids	−	−	+	+	+
11.	Lignin	−	−	−	+	+
12.	Volatile oil	−	−	−	−	−

(+) Indicates presence, (-) indicates absence. *P. bicalyculata: Peristrophe bicalyculata*

LPO assay

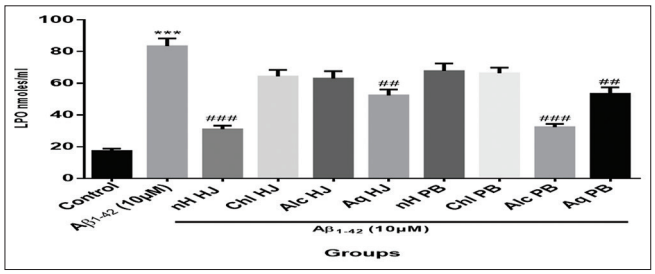

Fig. 1: Effect of different extracts of *Hydrocotyle javanica* and *Peristrophe bicalyculata* on lipid peroxide level in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism Version 6.0. Values are represented as Mean±SEM, superscript ***denotes p<0.001 versus control, ###denotes p<0.001; ##denotes p<0.01; #denotes p<0.05 versus $A\beta_{1-42}$, respectively. (Aβ: Amyloid beta; nH: n-Hexane, Chl: Chloroform; Alc: Alcohol; Aq: Aqueous)

NO assay

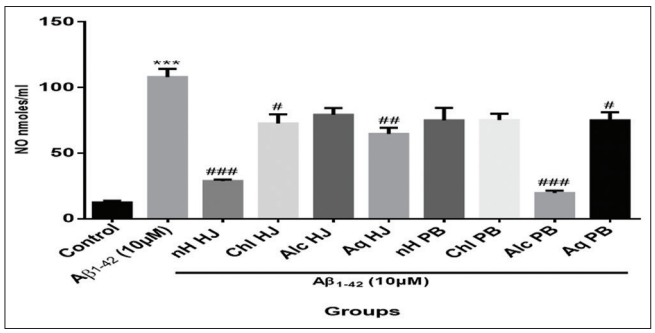

Fig. 2: Effect of different extracts of *Hydrocotyle javanica* and *Peristrophe bicalyculata* on nitric oxide level in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism Version 6.0. Values are represented as Mean±SEM, superscript ***denotes p<0.001 versus control, ###denotes p<0.001; ##denotes p<0.01; #denotes p<0.05 versus $A\beta_{1-42}$, respectively. (Aβ: Amyloid beta; nH: n-Hexane, Chl: Chloroform; Alc: Alcohol; Aq: Aqueous)

GSH assay

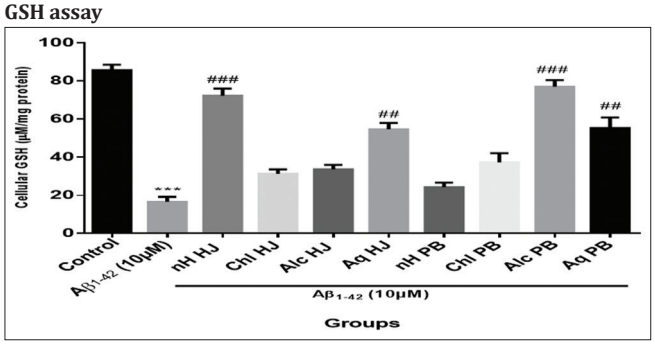

Fig. 3: Effect of different extracts of *Hydrocotyle javanica* and *Peristrophe bicalyculata* on cellular glutathione level in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism Version 6.0. Values are represented as Mean±SEM, superscript ***denotes p<0.001 versus control, ###denotes p<0.001; ##denotes p<0.01; #denotes p<0.05 versus $A\beta_{1-42}$, respectively. (Aβ: Amyloid beta; nH: n-Hexane, Chl: Chloroform; Alc: Alcohol; Aq: Aqueous)

SOD

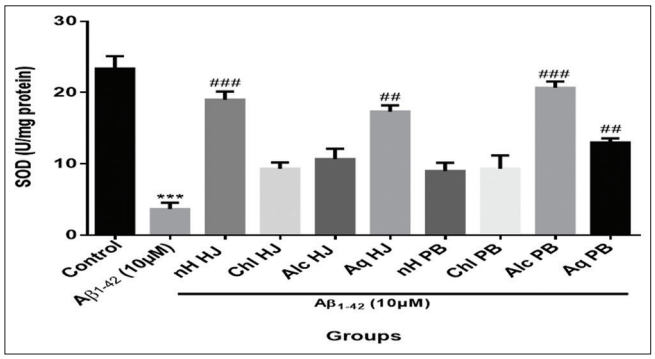

Fig. 4: Effect of different extracts of *Hydrocotyle javanica* and *Peristrophe bicalyculata* on superoxide dismutase level in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism Version 6.0. Values are represented as Mean±SEM, superscript ***denotes p<0.001 versus control, ###denotes p<0.001; ##denotes p<0.01; #denotes p<0.05 versus $A\beta_{1-42}$, respectively. (Aβ: Amyloid beta; nH: n-Hexane, Chl: Chloroform; Alc: Alcohol; Aq: Aqueous)

CAT

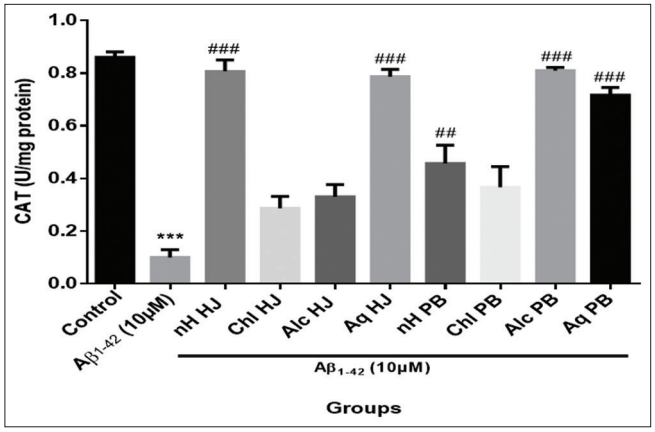

Fig. 5: Effect of different extracts of *Hydrocotyle javanica* (HJ) and *Peristrophe bicalyculata* (PB) on catalase level in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism Version 6.0. Values are represented as Mean±SEM, superscript ***denotes p<0.001 versus control, ###denotes p<0.001; ##denotes p<0.01; #denotes p<0.05 versus $A\beta_{1-42}$, respectively. (Aβ: Amyloid beta; nH: n-Hexane, Chl: Chloroform; Alc: Alcohol; Aq: Aqueous)

DISCUSSION

Pharmacognostical parameters are the first step to evaluate the distinctive character and to establish the identity and quality of crude drugs. In this research work, the phytochemical analysis shows the presence of various secondary metabolites such glycosides, phytosterol, saponins, alkaloids, carbohydrates, flavonoids, tannins, protein, and amino acid which are potential for further studies of antimicrobial and anti-inflammatory. Different extracts of *H. javanica* Thunb. and *P. bicalyculata* (Retz.) were evaluated for *in vitro* antioxidant activity using LPO assay, NO assay, GSH assay, SOD, and CAT. Among eight different extracts, n-Hexane *H. javanica* extract and alcoholic *P. bicalyculata* extract showed more potent *in vitro* antioxidant activity.

CONCLUSION

The extraction of the crude drug of *H. javanica* and *P. bicalyculata* was done successively by the Soxhlet method using petroleum ether, chloroform, alcohol, and water.

The preliminary phytochemical screening of the different extracts of two medicinal plants was performed and it was found to be the presence of glycosides, phytosterol, saponins, alkaloids, carbohydrates, flavonoids, tannins, protein, and amino acid. From the phytochemicals, results obtained it can be said that it is useful in the detection of bioactive principles which may lead to drug discovery and development.

The result of the present study suggests for the further investigation that the best results of antioxidant activity were n-Hexane *H. javanica* extract and alcoholic *P. bicalyculata* extract. Thus, the both extracts showed more potent antioxidant activity.

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AUTHORS' CONTRIBUTIONS

The corresponding author performed a collection of plants, extraction, experimental work, and wrote the manuscript and analyzed the data. The coauthor helped in evaluating the final manuscript. All the authors have read and agreed on the final approval of the manuscript.

CONFLICTS OF INTEREST

The author's declared that they have no conflicts of interest in publishing this research article.

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