SCREENING OF PHYTOCHEMICAL CONTENT AND IN VITRO BIOLOGICAL INVESTIGATION OF CANTHITUMDICOCUM (GAERTN.) AND AMISCHOPHACEULUS AXILLARIS (L.)

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ABSTRACT

Objective: The objective of the study was to study the pet ether, ethyl acetate, and ethanol leaf extracts of Canthium dicocum and Amischophaceulus axillaris for anthelmintic activity and antihypertensive activity.

Methods: The antihypertensive activity was carried out by employing a colorimetric assay based on the hydrolysis of Histidyl-Hippuryl-Leucine and anthelmintic activity carried out against Indian earthworm Pheritimaposthuma.

Results: The pet ether leaf extract both the plants exhibited the maximum antihypertensive activity with a percent inhibition of 64.82 for C. dicocum (Gaertn.) and 84.12 for A. axillaris (L.) as compared with Captopril showing percent inhibition 85.37 and for anthelmintic activity, it is found that ethanol extract of C. dicocum and ethyl acetate extract of A. axillaris exhibited significant activity against the standard drug albendazole.

Conclusion: This study investigated the potential of C. dicocum and A. axillaris as a new source against the antihypertensive activity. The outcome of anthelmintic activity revealed that the ethyl acetate and ethanol extracts exhibited a considerable amount of anthelmintic activity, which is mainly due to the active phytoconstituents present in the extracts.

Keywords: Antihypertensive, Anthelmintic, Canthium dicocum (Gaertn.), Amischophaceulus axillaris (L.), Angiotensin-Converting Enzyme inhibitors.

INTRODUCTION

Hypertension is nowadays common among people of all age groups. It does not give any early warning symptoms, but it exerts more load on heart and blood vessels due to which it is termed as a silent killer. Immense importance has been given to the study of hypertension with the development of a realistic method to measure it in the past century due to constant change in the lifestyle of people. Physicians have been working tirelessly to ascertain the relation between high blood pressure and risk of failure of heart, kidney, and even causes a stroke. Some early attempts in 1930s and 40s, including surgical procedures involving cutting nerves to blood vessels, inducing high fever and reducing sodium content in diets. Few case studies have yielded significant results proving the treatments are effective in lowering blood pressure and improving outcomes with minor setbacks. One of the proved methods of treatment of hypertension apart from drugs is by improving lifestyle and standard of living. The method of treatment as associated with dietary and lifestyle measures considerably reduces the arterial pressure thereby mitigates cardiovascular morbidity and mortality [1-4]. Search for new drugs, mainly from cheap and reliable natural products, and mainly plants are of significant interest in the development of more efficient and better-tolerated drugs. Therefore, it is relatively essential to study the inhibition of angiotensin-converting enzyme (ACE) to prevent and manage hypertension. Macroparasitic disease caused by parasitic worms that are visible to naked eye affecting humans as well as other animals wherein a part of the body or an organ is infected by the worm is known as helminths. Presently, helminthiasis is one of the common agents of infection rampant prevailing in developing as well as underdeveloped countries. The spread of helminthiasis which is a significant contributor to global diseases is worsened by prevailing malnutrition, pneumonia, anemia, and eosinophilia in underdeveloped countries [5] due to the nonavailability of basic health infrastructure and medically trained personnel to handle the situations. Helminthiasis is rarely fatal but is a major cause of morbidity [6]. The medicines available in the market which are chemically synthesized are not effective up to the mark and in some cases have developed resistance thereby causing recurrences of the diseases. Thus medicinal plants which are rich in botanical anthelmintics [7,3] serve as an alternate source for the development of more effective and less toxic medicines which has encouraged further research and development in analyzing new plant-derived medicines.

Canthium dicocum (Gaertn.), the Ceylon boxwood also known as Bellachi in Kannada, belongs to the family Rubiaceae [8]. In India, its bark is used for fever and decoction of the root is used internally for diarrhea. Bark powder with sesame oil is used in rheumatic pain [9,10]. The plant is proved for its anti-inflammatory [11], antidiabetic, and nephroprotective activity [12].

Amischophaceulus axillaris (L.) is a species of perennial plants in the family Commelinaceae commonly called Negilu there in Karnataka. It is native to the Indian Subcontinent, southern China, South East Asia, and Northern Australia. It grows in monsoon forest, woodland, and wooded grassland. Traditionally plant is used for anti-inflammatory, antiparasitic, and antifungal property. In India, leaves are used for the treatment of typanitis and as food for pigs [13].

METHODS

The C. dicocum (Gaertn.) leaves were collected in the month of June-July in Hosnagar (T), Fig. 1 Shimoga district, Karnataka, and A. axillaris (L.) Fig. 2 leaves were collected in the month of July-August in Agumbe region, Shimoga district, Karnataka. Both the plants were authenticated and deposited in the Department of Botany Kuvempu University, Shankaraghatta, with voucher number KUAB4688 for C. dicocum (Gaertn.) and KUAB4687 for A. axillaris (L.). The collected plant material was shade dried and coarsely pulverized. The pulverized plant material was subjected to the hot method of extraction using Soxhlet extractor. The extraction method was carried out using numerous solvents, namely, pet ether, ethyl acetate, and ethanol per their
increasing polarity. The obtained extract was filtered and evaporated to dryness under reduced pressure in a rotary vacuum evaporator.

**Qualitative phytochemical screening**

All the extracts were subjected to preliminary phytochemical analysis using the standard procedure to identify the various phytoconstituents [14].

**Antihypertensive activity**

The plant extract was tested at three concentrations dissolved in assay buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) HEPES buffer containing 0.3M NaCl and 10 μM Zinc Sulfate) containing 20 μl of kidney cortex plasma membranes (ACE enzyme source) and 1 mM Hippuryl-His-Leu as substrate. The extracts were incubated with the enzyme for 10 min at 37°C. The substrate was added to the reaction mixture and incubated for 45 min at 37°C. The reaction was terminated by the addition of 1 M HCl. The yellow color is developed by the addition of 100 μl of pyridine and 50 μl of benzene sulfonyl chloride and was measured at 410 nm in an ELISA Plate Reader (iMARK, BIORAD). The extract block availability of substrate to the enzyme and thereby cause enzyme inhibition, by indicating no formation of yellow color. The inhibition was represented in the form of a percentage over control. Captopril, a known ACE inhibitor was tested in this assay as a standard reference.

The inhibition activity (%) = \[(A_c - A_s)/(A_c - A_b)\] × 100

Where, A_c is the absorbance of the buffer (control), A_s is the absorbance of the reaction mixture (sample), A_b is the absorbance when the stoke solution was added before the reaction occurred (blank).

**Anthelmintic activity**

Indian adult earthworms *Pheritimaposthuma* were collected from earthworm rearing center, Dummalli, Shimoga (Karnataka). The worms were maintained in the cages with moderate temperatures. The worms were washed in water to remove dirt. The anthelmintic activity was evaluated on Indian adult earthworms *Pheritimaposthuma* due to its anatomical and physiological resemblances with the intestinal roundworm parasites of the human beings [17,18]. The activity was assessed using earthworms by the reported methods with small modifications [19]. The worms were washed to get rid of adhering materials and were sorted out for uniform size and length. The worms were kept in a 6% dextrose solution for acclimatization. The worms with modifications [19]. The worms were washed to get rid of adhering materials and were sorted out for uniform size and length. The worms were kept in a 6% dextrose solution for acclimatization. The worms with normal motility of length having 3–5 cm and 0.1–0.2 mm in width were used for the experiment. All the worms of equal size were divided into 11 groups and each group contains three worms. I group was treated with vehicle (1% Tween-80 in normal saline) served as control, II group is treated with albendazole (Standard) 10 mg/ml, and III – XI groups were treated with different concentrations (20, 40, and 60 mg/ml in normal saline containing 1% Tween-80) of all the three extracts. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the normal group did not survive in the saline. Death was concluded when the worm lost its motility followed by the fading of their body color. The experiment was carried out in triplicate for each group and data were statistically analyzed.

**RESULTS**

Results showed pronounced activity for pet ether extract with percent inhibition of 64.82 for *C. dicoccum* (Table 1, Fig. 3) and 84.12 for *A. axillaris* (Table 2, Fig. 4). These activities are comparable with the activity of the positive control, Captopril, which has a percent inhibition of 85.37. The preliminary evaluation of the crude extracts of *C. dicoccum* and *A. axillaris* showed that both the plants are a potential source of bioactive compounds that can inhibit the activity of ACE. The pet ether extract block availability of substrate to the enzyme and thereby cause enzyme inhibition, by indicating no formation of yellow color. The inhibition activity was calculated using the following equation

Inhibition activity (%) = \[(A_c - A_s)/(A_c - A_b)\] × 100

Where, A_c is the absorbance of the buffer (control), A_s is the absorbance of the reaction mixture (sample), A_b is the absorbance when the stoke solution was added before the reaction occurred (blank).

**Table 1: Antihypertensive activity of various solvent extracts of Canthium dicoccum (Gaertn.) leaves with standard**

| Samples | Concentration | O.D. | OD-Blank | % ACE inhibition |
|---------|---------------|------|----------|-----------------|
| Blank   |               | 0.405| 0.580    |                 |
| Control |               | 0.985|          |                 |
| A       | 20 μg         | 0.773| 0.368    | 36.55±1.194     |
|         | 40 μg         | 0.740| 0.335    | 42.24±0.000     |
|         | 60 μg         | 0.687| 0.282    | 50.97±0.583     |
| B       | 80 μg         | 0.680| 0.275    | 56.26±0.119     |
|         | 100 μg        | 0.699| 0.204    | 64.87±0.895     |
|         | 20 μg         | 0.639| 0.234    | 59.65±0.796     |
|         | 40 μg         | 0.652| 0.247    | 57.41±0.298     |
| C       | 60 μg         | 0.798| 0.334    | 33.79±5.97*     |
|         | 80 μg         | 0.772| 0.367    | 36.72±0.896     |
|         | 100 μg        | 0.758| 0.353    | 39.13±0.497     |
|         | 20 μg         | 0.786| 0.371    | 36.03±5.97*     |
|         | 40 μg         | 0.844| 0.439    | 24.31±0.696**   |
| D       | 60 μg         | 0.954| 0.549    | 5.34±5.97**     |
|         | 80 μg         | 0.974| 0.569    | 2.01±0.897**    |
|         | 100 μg        | 1.021| 0.616    | 0.00±0.997**    |
|         | 10 nM         | 0.506| 0.305    | 33.41±0.756     |
|         | 15 nM         | 0.412| 0.211    | 51.96±1.134     |
|         | 20 nM         | 0.268| 0.067    | 85.37±0.988     |

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett’s test and differences between means were regarded significant at p<0.05*, p<0.01**, C. dicoccum: Canthium dicoccum. SEM: Standard error of the mean, ANOVA: Analysis of variance, OD: Optical density.
extract of both the plants was found to have the highest ACE inhibitory activity. Further purification studies will be carried out to identify the bioactive compounds responsible for the observed activity.

For anthelmintic activity, the time is taken for mean paralysis and means the death of the earthworms are tabulated in Tables 3 and 4. The main effect of albendazole on the worm is to cause a flaccid paralysis that effects in exclusion of the worm by peristalsis. The results of the present study revealed that all the tested extracts of *C. dicoccum* (Gaertn.) and *A. axillaris* (L.) have anthelmintic activity in dose-dependent method giving the shortest time of paralysis and death of worms. The results showed that the ethanol extract of *C. dicoccum* (Gaertn.) and ethyl acetate extract of *A. axillaris* (L.) exhibited considerable anthelmintic activity at a concentration of 60 mg/ml by causing the death of worms in lesser time. The anthelmintic activity of all the extracts was comparable to that of the standard drug albendazole. Preliminary phytochemical screening of crude extracts of *C. dicoccum* and *A. axillaris* revealed that the presence of various phytochemical constituents is tabulated in Tables 5 and 6.

**DISCUSSION**

A reference study showed that pet ether extract of *C. dicoccum* contains high concentration of phenolic compounds [20]. The free hydroxyl groups of phenolic compounds are the structural moieties that chelate zinc ions in the active site of ACE thereby rendering ACE inactive [21]. The present results showed that both *C. dicoccum* and *A. axillaris* showed a high concentration of phenolic groups, which is the major moiety to rendering ACE inactive. Some of the secondary metabolites that could be responsible for the observed *in vitro* ACE inhibitory activity are steroids, flavonoids, glycosides, and hydrolyzable tannins. The results of the phytochemical screening revealed the presence of alkaloids, terpenes, flavonoids, tannins, anthraquinones, and saponins in *C. dicoccum* extract [20]. Elbl and Wagner developed one of the earliest

![Fig. 2: Amischophacelus axillaris (L.) plant](image)

| Table 2: Antihypertensive activity of various solvent extracts of *Amischophacelus axillaris* (L.) leaves with standard | Samples | Concentration (mg/ml) | O.D. | OD-Blank | % ACE inhibition |
|-----------------------------------------------------------|---------|-----------------------|------|----------|-----------------|
| Blank                                                     | 0.308   | 0.914                 | 0.693| 68.398±0.416 |
| Control                                                   | 0.527   | 0.219                 | 66.378±0.831 |
| A                                                         | 0.498   | 0.190                 | 84.993±0.833 **|
| 80 µg                                                     | 0.412   | 0.104                 | 72.58±0.166  |
| 100 µg                                                    | 0.418   | 0.110                 | 84.127±0.249 **|
| 20 µg                                                     | 0.576   | 0.268                 | 52.236±0.333 |
| 40 µg                                                     | 0.544   | 0.236                 | 44.589±0.333 |
| B                                                         | 0.534   | 0.226                 | 45.02±0.416  |
| 60 µg                                                     | 0.512   | 0.204                 | 47.474±0.916 |
| 80 µg                                                     | 0.501   | 0.193                 | 49.495±0.249 |
| 100 µg                                                    | 0.639   | 0.331                 | 61.327±0.166 |
| 40 µg                                                     | 0.699   | 0.384                 | 65.945±0.499 |
| C                                                         | 0.699   | 0.384                 | 67.38±0.499  |
| 80 µg                                                     | 0.672   | 0.364                 | 70.56±0.416  |
| 100 µg                                                    | 0.658   | 0.350                 | 72.150±0.831 |
| 10 nM                                                     | 0.506   | 0.305                 | 33.410±0.756 |
| D                                                         | 0.412   | 0.211                 | 51.966±1.134 |
| 15 nM                                                     | 0.268   | 0.067                 | 85.37±0.883  |

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at p<0.05*, p<0.01**, *A. axillaris*(L.): *Amischophacelus axillaris* (L.), SEM: Standard error of the mean, ANOVA: Analysis of variance, OD: Optical density

| Table 3: Anthelmintic activity of various solvent extracts of *Canthium dicoccum* (Gaertn.) leaves with standard |
|-----------------------------------------------------------|---------|-----------------------|-----------------|
| Treatment groups                                        | Concentration (mg/ml) | Mean paralysis time (min)±SEM | Mean death time (min)±SEM |
| Control/Vehicle                                         | 7.13±0.008           | 13.09±0.039             |
| Standard                                                | 14.13±0.20           | 22.13±0.014             |
| A                                                       | 12.12±0.023          | 20.10±0.017             |
| B                                                       | 10.08±0.017          | 20.08±0.020             |
| C                                                       | 12.14±0.012          | 24.1±0.008              |
| 20                                                      | 10.06±0.026          | 22.13±0.026             |
| 20                                                      | 10.10±0.015          | 18.12±0.011             |
| 20                                                      | 12.06±0.008          | 15.10±0.008             |
| 20                                                      | 9.09±0.020           | 18.14±0.014             |
| 20                                                      | 9.08±0.008           | 16.15±0.017             |

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at p<0.05*, p<0.01**, *C. dicoccum: Canthium dicoccum*. SEM: Standard error of the mean, ANOVA: Analysis of variance
Fig. 3: Antihypertensive activity of *Canthium dicoccum* (Gaertn.) of various solvent extracts in angiotensin-converting enzyme inhibitors method. AP- pet ether extract, AE- ethyl acetate extract, Aet- ethanol extract, STD: Standard, Standard = Captopril in 10 nM, 15 nM, 25 nM

Fig. 4: Antihypertensive activity of *Amischophacelus axillaris* (L.) of various solvent extracts in angiotensin-converting enzyme inhibitors method. AP- pet ether extract, AE- ethyl acetate extract, Aet- ethanol extract, STD – standard. Standard = Captopril in 10 nM, 15 nM, 25 nM
Table 4: It shows anthelmintic activity of different extracts of *Canthium dicoccum* (Gaertn.) and *Amischophacelus axillaris* (L.) leaves with standard

| Treatment groups | Concentration (mg/ml) | Mean paralysis time (min)±SEM | Mean death time (min)±SEM |
|------------------|-----------------------|------------------------------|--------------------------|
| Control/Vehicle  |                       | ---                          | ---                      |
| Standard         | 10                    | 7.13±0.008                   | 13.09±0.039              |
|                  | 20                    | 18.09±0.005                  | 22.14±0.012              |
|                  | 40                    | 15.10±0.003                  | 20.09±0.005              |
|                  | 60                    | 13.14±0.014                  | 18.11±0.008              |
| A                | 20                    | 17.11±0.008                  | 23.08±0.005              |
|                  | 40                    | 10.08±0.014                  | 20.11±0.020              |
|                  | 60                    | 9.05±0.012                   | 15.05±0.026              |
| B                | 20                    | 16.14±0.015                  | 21.14±0.028              |
|                  | 40                    | 15.03±0.173                  | 20.01±0.046              |
|                  | 60                    | 13.02±0.023                  | 18.02±0.00               |

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett’s test and differences between means were regarded significant at p<0.05*, p<0.01**. *A. axillaris* (L.); *Amischophacelus axillaris* (L.); SEM: Standard error of the mean, ANOVA: Analysis of variance

Table 5: Phytochemical screening of various solvent extracts of *Canthium dicoccum* (Gaertn.) leaves

| Phytoconstituents | Pet. ether extract | Ethyl acetate extract | Ethanol extract |
|-------------------|--------------------|-----------------------|-----------------|
| Alkaloids         | -ve                | +ve                   | +ve             |
| Steroids          | -ve                | +ve                   | +ve             |
| Carbohydrates     | +ve                | +ve                   | +ve             |
| Flavonoids        | -ve                | +ve                   | -ve             |
| Phenolics/Tannins | +ve                | +ve                   | +ve             |
| Saponins          | +ve                | +ve                   | +ve             |
| Glycosides        | -ve                | +ve                   | +ve             |
| Coumarin          | -ve                | +ve                   | +ve             |

Further investigations on the isolation of active compounds present in the extracts and in vivo studies are essential to identify a potential chemical entity for clinical use in the treatment of hypertension and further related cardiovascular disorders. This study investigated the potential of *C. dicoccum* and *A. axillaris* as a new source against ACE.

**CONCLUSION**

The present study suggests that the leaves of *C. dicoccum* and *A. axillaris* possess ACE inhibitory that might be helpful in treating hypertension.

Table 6: Phytochemical screening of various solvent extracts of *Amischophacelus axillaris* (L.) leaves

| Phytoconstituents | Pet. ether extract | Ethyl acetate extract | Ethanol extract |
|-------------------|--------------------|-----------------------|-----------------|
| Alkaloids         | -ve                | +ve                   | +ve             |
| Steroids          | -ve                | +ve                   | +ve             |
| Carbohydrates     | +ve                | +ve                   | +ve             |
| Flavonoids        | +ve                | +ve                   | -ve             |
| Phenolics/Tannins | +ve                | +ve                   | +ve             |
| Saponins          | +ve                | +ve                   | +ve             |
| Glycosides        | +ve                | -ve                   | +ve             |
| Coumarin          | -ve                | +ve                   | +ve             |

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