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**Sudharameshwari K**
Assistant Professor,
Department of PG Biochemistry,
V.V. Vanniaperumal College for Women, Virudhunagar,
Tamil Nadu, India

**Rekha I**
PG Biochemistry Student,
V.V. Vanniaperumal College for Women, Virudhunagar,
Tamil Nadu, India

**Salini R**
Assistant Professor,
Department of PG Biochemistry,
V.V. Vanniaperumal College for Women, Virudhunagar,
Tamil Nadu, India

**Correspondence**
**Sudharameshwari K**
Assistant Professor,
Department of PG Biochemistry,
V.V. Vanniaperumal College for Women, Virudhunagar,
Tamil Nadu, India

# Bioactive potential of *Cardiospermum halicacabum* and *Butea monosperma* leaf extract in combination

**Sudharameshwari K, Rekha I and Salini R**

**Abstract**
This study aimed to evaluate the combinatorial effect of *Cardiospermum halicacabum and Butea monosperma* extract for antioxidant and antimicrobial activity. Use of herbal drugs plays a vital role in the treatment of various diseases. There is evidence that the constituents of a local plant *C. halicacabum* and *B. monosperma* have several medicinal properties. In this study, the leaves of *C. halicacabum* and *B. monosperma* were shade dried and used to obtain different solvent extracts (aqueous, ethanol and methanol) of the respective plants. Light green, dark green and brown colour powders were observed in the aqueous, methanol and ethanol extract of *C. halicacabum* and *B. monosperma* respectively. This extract was evaluated for phytochemicals, antioxidant and antimicrobial activity. The phytochemical screening of *C. halicacabum* and *B. monosperma* leaves (Aqueous, Ethanol, Methanol) extract revealed the presence of phytoconstituents such as flavonoids, protein, alkaloids, terpenoids, saponin and glycosides. The number of phytoconstituents is much higher in the aqueous extract of *C. halicacabum* and *B. monosperma* leaves when compared to ethanol extract and methanol extract. So the aqueous extract is one of the best extracts to solubilize almost most of the compounds followed by ethanol extract. Antimicrobial activity of *B. monosperma* and *C. halicacabum* extracts were checked against different test organisms such as *Pseudomonas aeruginosa, Protease vulgaris, Vibrio cholerae, Shigella Sp.* and *Citrobacteria* using agar diffusion method. The aqueous extract showed maximum inhibitory action against most of the microorganisms. The combination of *C. halicacabum* and *B. monosperma* extracts act synergistically and showed maximum zone of inhibition. Hence, the combinations of these two plant extracts were found to be a good antimicrobial agent to combat disease caused by multi drug resistant pathogens.

**Keywords:** Antioxidant activity, *Butea monosperma*, *Cardiospermum halicacabum*, combinatorial effect, antimicrobial activity

## Introduction
Eco-friendly plant based commodities has recently been given consideration for the prevention and treatment of various human infection including microbial disease throughout the world [1] and employment of plants in ethno medicine is on rise worldwide [2]. Recently scientific interest in medicine plant has burgeoned due to increased efficiency of plant derived drugs and raising concern about the side effect of modern medicines [1]. Several hundreds of plants in India are known to have the potential to treat many diseases and one of those popular are *Cardiospermum halicacabum* and *Butea monosperma*. It is traditionally used for the treatment of inflammatory diseases [3]. *B. monosperma* is commonly known as *flame of forest* belongs to the family Fabaceae [4]. It is generally to grow gregariously on open grasslands and scattered in mixed forest, plantations can be raised both on irrigated and dry lands [5]. It also possess antitumor, antiulcer, wound healing and antimicrobial activities [6, 7]. *Cardiospermum halicacabum* of the family Sapindaceae has been used in Ayurveda and folk medicine for a long time in the treatment of rheumatism [8]. Extract of this plant have been reported to contain different triterpenoids, glycosides, and a range of fatty acids [9, 10, 11]. The leaves of the plants were reported to have antioxidant and antibacterial activities [12]. Therefore, this study was indented to determine the combinatorial effect of *C. halicacabum* and *B. monosperma* for antioxidant and antimicrobial activity.

## Materials and methods
### Extraction of Plant Material
The plant *C. halicacabum* and *B. monosperma* were collected from Virudhunagar, Tamil Nadu, India. The plants were authenticated by a botanist Dr. B. Karunaiselvi, Assistant Professor, Department of Botany, V.V. Vanniaperumal College for Women, Virudhunagar,

Tamil Nadu, India. The leaves of the plants were washed several times with distilled water. 25 g of shade dried leaves of both plants were taken and grounded using Mortar & Pestle. 25 g of powdered material was added to 40 ml of respective solvents (distilled water, methanol ethanol) individually and kept in shaker for 72 h. The extracts were filtered by Muslin cloth and these extracts were dried at room temperature and the obtained extract was stored in dry box.

Qualitative Phytochemical Analysis

Aqueous, ethanol and methanol extracts were subjected to qualitative phytochemical analysis to identify the presence of phytoconstituents such as tannin, terpenoids, alkaloids, glycosides, steroids, flavonoids, phenol, protein, carbohydrate, quinine, anthroquinone and saponins [13, 14, 15].

Quanititative Phytochemical Analysis

The amount of protein [16, 17]. Phenol [18]. Tannins [19]. Alkaloids, flavanoids [20]. Ascorbic acid [21]. And terpenoids present in the different extracts were estimated.

**Antioxidant Activity**

Antioxidant activities of the different extracts were determined by using different standard methods. The methods used are DPPH photometric assay [22]. Superoxide radical scavenging activity [23]**.** Ferric reducing power determination [24]. Reducing power activity, catalase activity and thiobarbutric acid reactive substances assay.

**Antimicrobial Activity**

Microbial strains

Authentic pure cultures of bacteria namely *Pseudomonas aeruginosa, Shigella Sp., Citrobacteria, Proteus vulgaris* and *Vibrio cholerae* were obtained from microbiology laboratory V.V. Vanniaperumal College for Women, Virudhunagar, Tamil Nadu, India.

**Agar Well Diffusion Method**

The extracts were screened against five bacterial pathogens in triplicate. Antimicrobial activity was carried out using agar well diffusion method. The bacterial strains were inoculated in Nutrient broth and inoculated for 24 hours before used for antimicrobial assay. Sterile Muller Hinton Agar (Hi-Media) plates (MHA) were prepared and allowed to set. The cultures to be screened were swab streaked on MHA plates. The wells are made in MHA plates using corn borer (7mm diameter) and the extract (100µl) was loaded in the wells. Ethanol, methanol, water were used as negative control. Ampicillin (antibiotic) was used as standard control. The plates were incubated at $37^0$c for 24 hours. After incubation, the zone of inhibition was measured in cm.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Microsoft Excel 2010 statistical package was used for all analyses.

**Results and discussion**

Pharmaceutical research conducted has shown that the natural products are a potential source of novel molecules for the drug development. The collection of plant materials based on traditional uses had a higher degree of positive results. The colour consistency of different solvent extract of *Cardiospermum halicacabum* and *Butea monosperma* was shown in table 1 and fig 1.

**Table 1:** The colour consistency of different solvent extract of *C. halicacabum and B. monosperma*

| Plants | Extract | Colour | Consistency |
|---|---|---|---|
| *C. halicacabum* + *B. monosperma* | Aqueous | Light Green | Powder |
| | Ethanol | Dark Green | Powder |
| | Methanol | Brown | Powder |

**Fig 1:** The colour consistency of various extract of *C. halicacabum and B. monosperma*

**Qualitative Phytochemical Test Analysis:**

The phytochemical screening of combination of *C. halicacabum* and *B. monosperma* leaves (Aqueous, Ethanol, Methanol) extract revealed the presence of phytoconstituents such as flavonoids, protein, alkaloids terpenoids, saponins, glycosides (Table 2). Aqueous extract of *C. halicacabum and B. monosperma* leaves showed the presence of most phytoconstituents compared to ethanol extract and methanol extract. So the aqueous extract is one of the best extract followed by ethanol extract. The anti- arthritis effect of aqueous extract of *C. halicacabum and B. monosperma* leaves could be due to the presence of saponin, terpenoids, flavanoids, alkaloids and anthroquinone. The preliminary phytochemical analysis of *B. Monosperma* leaves shows the absence of alkaloids, flavonoids, glycosides, tannins in aqueous extracts [25]. But in our combinatorial analysis of plant extracts (aqueous, ethanol, methanol) shows the presence of alkaloids, flavonoids, glycosides, tannins. One (or) more chemical present in individual plant extract Maluventhan Viji and Sangu Murugesan, 2010 [26]. Reported that terpenoids, flavaonoids and glycosides are absent in aqueous extract of *C. halicacabum* but in our study combination of both plant shows the presence of flavanoids, terpenoids & glycosides.

**Table 2:** Phytochemical Screening of various solvent extract of *C. halicacabum & B. monosperma*

| Phytochemical constituent | Aqueous extract of *C. halicacabum* + *B. monosperma* [AE] | Ethanol extract of *C. halicacabum* + *B. monosperma* [EE] | Methanol extract of *C. halicacabum* + *B. monosperma* [ME] |
|---|---|---|---|
| Alkaloids | Present | Present | Present |
| Tannin | Present | Present | Present |
| Saponin | Present | Present | Present |
| Terpenoids | Present | Present | Present |
| Flavonoids | Present | Present | Present |
| Anthraquinone | Present | Absent | Absent |
| Glycosides | Present | Present | Present |
| Phenol | Present | Present | Present |
| Protein | Present | Present | Present |

**Quantitative Analysis of Phytochemical Constituents:**

The medicinal value of plants lies in some substances that have a definite physiological action on the human body. Different phytochemical have been found to possess a wide range of activities, which may help in protection against chronic diseases [27]. More recently drug techniques have been applied to the standardization of herbal medicine sciences. Some of the phytoconstituents were quantitatively determined were shown in table 3.The result revealed that the aqueous leaves extract showed the higher amount of alkaloids, tannin, terpenoids, flavonoids, phenol and ascorbic acid than other extract. It has antimicrobial and antifungal properties, along with anti- inflammatory. In medicine, it is used as antioxidant, anticancer, anti- inflammatory & anti-arthritis. Phenol content was high in aqueous extract followed by methanol & ethanol extract. The presence of tannin in the leaves *C. halicacabum and B. monosperma* supports its strong use for healing of wounds, ulcers, hemorrahoids, frost-bites and burns in herbal medicine. The building block of protein was present. Flavonoids are widely distributed in plant, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals [28]. Higher amount phenol was present in the aqueous extract followed by methanol extract.

**Table 3:** Quantitative analysis of phytochemical constituents (mg/g) in the combination of *C. halicacabum and B. monosperma*

| Test phytochemical constituents | aqueous extract | ethanol extract | methanol extract |
|---|---|---|---|
| Alkaloids | 1.2±0.1 | 0.7±0.05 | 0.5±0.02 |
| Tannin | 1.03±0.05 | 0.5±0.01 | 0.3±0.1 |
| Terpenoids | 2.5±0.12 | 1.5±0.13 | 0.7±0.07 |
| Flavonoids | 6.68±0.15 | 5.04±0.1 | 3.75±0.05 |
| Protein | 0.32±0.1 | 3.75±0.11 | 0.1±0.01 |
| Phenol | 6.4±0.04 | 1.5±0.08 | 5.5±0.1 |
| Ascorbic acid | 1.7±0.02 | 1.0±0.01 | 0.7±0.2 |

Values are expressed as mean ± standard deviation (SD), n=3

**Antioxidant Activity**

In the present study, free radical scavenging activity of various extracts of *C. halicacabum and B. monosperma* leaves were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS [29]. Antioxidants due to their scavenging activity are useful for the management of those diseases [29]. The main function of an antioxidant is its ability to trap free radicals. The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was shown in table 4. These findings show that the various extracts of *C. halicacabum and B. monosperma* possess antioxidant activity. DPPH assay revealed that aqueous extract had the highest antioxidant activity [25%] comparable with vitamin C which was taken as positive control. From results of superoxide radical scavenging activity, it was found that aqueous extract [40%] showed high potent free radical scavenging activity. Ethanol [35%] and methanol [16%] extract also showed good activity in the decreasing percentage of inhibition. The FRAP assay is widely used in the evaluation of antioxidant components in dietary polyphenols [30]. Similar to our result, Ayswarya M and Sudha Rameshwari K, 2015 [31]. Reported that *Brasscia oleraceae* of various solvent extract also showed high antioxidant activity. According to a well-established thought, antioxidant activities of various vegetables, fruits, and natural plants are attributed to the contents of phenolic compounds [32]. In aqueous extract, more catalase activity is seen than followed by ethanol & methanol. Furthermore, reactive oxygen species seem to influence cell signaling pathways in ways that are only now being unraveled. It is estimated that half of the world's fruit and vegetable crops are lost due to postharvest deteriorative reactions [33]. Superoxide radical scavenging activity was shown by both curcuma and coffee bean extracts and was concentration dependent with an IC50 value of 0.4±0.14 and 0.52±0.18 mg /mL respectively [34]. In this plant, superoxide radical scavenging activity highest value (40mg/ml) and then least value methanol extract (16mg/ml). Antioxidant activities of aqueous, ethanol and methanol extract of *C. halicacabum and Butea monosperma* in combination were estimated by six different assays. The results proved that the overall antioxidant activity were highest in aqueous extract as compared to others.

**Table 4:** Antioxidant activities of *C. halicacabum and B. monosperma* in combination

| S. No | Antioxidant | AE | EE | ME |
|---|---|---|---|---|
| 1 | Superoxide Radical Scavenging Activity | 40% | 35% | 16% |
| 2 | Reducing Power Activity [RA] | 15% | 13% | 10% |
| 3 | Ferric Reducing Power Determination [FRAP] | 33% | 29% | 20% |
| 4 | Thiobarbutric Acid Reactive Substances Assay [TBARS] | 44% | 33% | 23% |
| 5 | Catalase | 73% | 65% | 52% |
| 6 | Diphenyl-2-picryl hydrazyl Assay [DPPH] | 25% | 16% | 15% |

AE - aqueous extract; EE – ethanol extract; ME – methanol extract

**Antimicrobial Activity**

Antibacterial activity of *B. monosperma and C. halicacabum* in combination were checked against different test organisms which are *P. aeruginosa, P. vulgaris, V. cholerae, Shigella Sp., Citrobacteria* were shown in table 5. The combined antibacterial property of leaves extract of *C. halicacabum and B. monosperma* was analyzed against bacterial pathogen using

ampicillin as control. *C. halicacabum and B. monosperma* as found to be very effective against *P. aeruginosa which* exhibited a maximum zone of inhibition with 1.5±0.1cm, 1.0±0.1cm, 1.0±0.2cm aqueous, ethanol, and methanol in *P. vulgaris, Citrobacteria, V. cholerae, Shigella Sp.* pathogen revealed a zone of inhibition ranges from 0.7cm to 1cm in different solvent extraction. Maximum inhibition was obtained against *P. aeruginosa* by aqueous extract and the second highest inhibition by ethanol followed by methanol. *P. aeruginosa* exhibited more inhibitory activity due to the role of phytoconstituents which is responsible for the action of permeability of peptidoglycan layer. Malpani M O *et al.*, 2012 [35]. Reported that methanol extract of *B. monosperma* does not inhibit the growth of *pseudomonas sp.* But in our combined plant methanol extract has the inhibitory growth against *Pseudomonas sp.* may be due to the presence of glycosides, terpenoids, flavonoids, saponins. Aqueous extract was found to be more potent antibacterial agent against *Citrobacteria* followed by ethanol. The zone of inhibition of methanol extract was found to be maximum against *P. vulgaris*.

**Table 5:** Antimicrobial screening of various solvent extract of *Cardiospermum halicacabum and Butea monosperma* in combination

| Microorganism | Zone of inhibition in diameter (in cm) | | | |
|---|---|---|---|---|
| | Aqueous extract | Ethanol extract | Methanol extract | Standard |
| *Pseudomonas aeruginosa* | 1.5±0.1 | 1.0±0.1 | 1.0±0.2 | 0.6±0.1 |
| *Citrobacteria* | 1.0±0.15 | 0.8±0.12 | 0.7±0.1 | 0.5±0.11 |
| *Protease vulgaris* | 1.0±0.14 | 0.7±0.05 | 0.8±0.11 | 0.4±0.03 |
| *Vibrio cholerae* | - | 0.5±0.1 | - | 0.8±0.15 |
| *Shigella* | 0.5±0.1 | - | - | 0.7±0.1 |

'- '= no zone formation; Values are expressed as mean ± standard deviation, n=3

The inhibition zone is nearly similar for ethanol and aqueous extract. *V. cholerae* showed a maximum zone of inhibition in ethanol. Aqueous and methanol extract has no inhibition activity. Similar results were reported by Venkatesan *et al* 2006 [36]. The difference in the observed activities of the various extracts may be due to varying degree of solubility of the active constituents in the solvents [37]. The antibacterial activity of aqueous and ethanol solvent extract revealed no activity against *V. cholerae* studied. Our results are supported by Deepan *et al.*, 2012 [38]. Observed the excellent antimicrobial activity of aqueous extract of *C. halicacabum* against Escherichia coli. According to Veerappan *et al.*, 2012 [39], the alcoholic extract of *C. halicacabum* has exhibited potent antimicrobial activity.

## Conclusion

Use of herbal drugs plays a vital role in the treatment of various diseases. Among the different solvent extract, aqueous extract showed significant results regarding antioxidant studies and antimicrobial activity. Studies were further extended to pharmacological studies.

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