

Evaluation of cow ark enhanced *Plectranthus Amboinicus* for the potential of antioxidant, antimicrobial, and larvicide potentials – in vitro

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ABSTRACT

Background: Current scientific investigations are receiving an increased interest in developing novel drugs from natural origin, specifically, herbal sources for Human health care. The prime objective of the investigation was designed to evaluate effects of cow ark (distillate), that has been reportedly, and believed to be, a bio enhancer, with a selected plant extract, *Plectranthus Amboinicus* and their impact, on the antioxidant, antimicrobial, antibiofilm formation and larvicide abilities, using in vitro animal models.

Methods: The antioxidant ability of cow ark, with extract of *Plectranthus Amboinicus*, by employing, DPPH method, to determine the scavenging behavior. Phytochemical composition was strictly screened, and reported. Antimicrobial activity was performed, using a well diffusion method. The Larvicide ability was determined by employing the protocol, described by WHO. The Anti biofilm formation was also examined under light and confocal laser microscopy.

Results: In the screening of phyto constituent composition, results revealed that Alkaloids, flavonoids, phenolic and terpenoids were detected in dominant. Antimicrobial ability was shown to be effective against Gram negative pathogens such as *E.Coli*. Developing bio film was arrested when treated with cow ark was reported. Larvicide effects of the bio enhancer in cow ark showed maximum 10 mg/ml. concentration against mosquito larvae of *Aedes aegypti*, indicating a candidate potential of larvicide action against mosquito larvae.

Conclusion: The present study concluded that cow ark with a plant extract showed a promising therapeutic effects to combat in the battle, against threatened infectious pathogens. Examined cow ark, served as a bio enhancer with *Plectranthus Amboinicus* to activate and improve potentials of cow ark bio molecules to offer effectiveness against disease curing biological actions.

Keywords: Bio enhancer, cow ark, Larvicide effects, anti microbial, *Plectranthus Amboinicus*.

1. INTRODUCTION

Cow ark is considered as an incredible and provocative part in Indian traditional medicine. In classical literature, *Bos indicus* was pronounced extensively and highlighted as "Mother of All". A mountain of evidence proved its effectiveness in the recent past, and has increased much attraction in the research, due to its ability has been explored, day by day in curing several diseases, exclusively or with combination of other potential herbs. Distillate of cow urine has been proved to be an excellent anti microbial activity against a spectrum of classes of Gram Negative and Gram positive clinical pathogens and Multi Drug Resistant bacteria [1] Moreover, a number of research was shown to have, its combined characteristics with anti fungal ,anti cancer and anti microbial drugs as a bio catalyst [2], Cow ark retracts the dreaded diseases and increase resistance ability in human body was also reported [3].

In recent times, destroying the sources of infectious organisms, in the control of mosquito prone diseases using insightful phytochemical based medicines with eco friendly manner is increasing, attributing, its less toxic to the human and his desirable species. A great interest exists in developing bio based insecticides in the field of entomological research. Scavenging ability of phytoconstituents or secondary metabolites exhibited a high potential in reducing effects of dreadful diseases. Use of plant sources is a promising, effective and easily curable against health related concern in tropical countries, including India [4]. Studies on the phytochemical with anti parasite potential, using cow ark, as a bio enhancer has been described in the literature, is scanty. Therefore, in the present study, an effort has been taken to evaluate the cow ark as a bio enhancer, with a selected medicinal plant extract, *Plectranthus Amboinicus* on the antioxidant and antimicrobial and anti bio film formation in pathogens, and larvicide potentials using *in vitro*

2. MATERIALS AND METHODS

Materials

Plant material, *Plectranthus Amboinicus* was collected from homestead plantations, available from abutting villages of Karaikudi, Tamil Nadu, with an intensified survey and got identification and authenticity. Plant leaves were dried in sunshade and in dark place. Well dried leaves were selected to grind, using mechanical apparatus, converted into powder form. Two hundred grams were weighed and taken as samples, to determine the biological enhancing activity along with cow ark.

Collection of Cow urine sample

Cow urine samples were collected from a healthy Indian origin cow (*Bos indicus*) 5-10 years old, using a sterile wide mouth flask, collected under standard hygienic condition. It is to note that infected and conceived cows urine was ignored in our study. Collection was achieved in early onset of day, after discarding first flow. Obtained samples were brought to the laboratory in an ice box with tight lids. In our laboratorial condition, it was filtered by passing into 0.22 urn units of filtration that were kept under -20°C for conducting a series of analytical examinations.

Formulation of Cow Ark with *Plectranthus Amboinicus* extract

A quantity of 10gm of plant material was used to make filtrate extract by adding 100ml of cow urine and aged for 15 days. After a stipulated period, the mixer was screened

with muslin cloth and Whatman No.1 Filter paper and supernatant was kept in the cool condition, using an IFA refrigerator as demonstrated by Edwin J. et al., (2008) [5].

Qualitative phytochemical examination

Qualitative analysis on the formulated extract was carried on to determine the occurrence of communal phytoconstituents such as flavonoids, glycosides, steroids, glycosides saponins, phenols, resins, tannins, Ferrous chloride and Terpenoids by following a standard protocol as described by Tamilarasi, et al. (2012) [6].

Estimation of Antimicrobial potential

In the present investigation, effectiveness in the antimicrobial ability of bio-enhancer such as combined Cow Ark, with plant extract of *Plectranthus Amboinicus* was determined, against two Gram positive strains such as *Staphylococcus aureus* and *Bacillus subtilis* and two Gram negative strains such as *Pseudomonas aeruginosa* and *E.Coli* were chosen for the trial. The Agar well diffusion technique was employed in the study that involved fresh Muller Hinton Agar (MHA) media plates. The wells were filled with varied concentrations of test solutions (cow ark with extract of *Plectranthus Amboinicus*) such as 25 UL, 50 UL, 75 UL and 100 UL/mg were determined. The examined solutions were allowed to intrude the plant extracts with Cow Ark into the prepared medium. They were incubated at 37⁰ C for 24 hours. The zone of inhibition was scaled using meter ruler [7].

Antioxidant estimation -DPPH measure

In this study, we investigated antioxidant potential of cow ark with selected plant extract such as *Plectranthus Amboinicus* was determined by adopting DPPH measure, by following Blois (1958) [8] protocol with slight alteration based on laboratorial compatibility. 1 ml of cow ark blended with bio enhancers was taken and 1 ml of ethanol solution contains DPPH free radical was added. The concentration regimen was maintained as 0.041 mm, as described by Braca et al (2001) [9]. The test solution was shaken tediously and aged for 10 minutes. The absorbance was noted at 517 nm in Spectroscopy.

Larvicide efficacy determination

The larvicidal potential of cow ark with *Plectranthus Amboinicus* was examined by employing the World Health Organization (WHO) protocol with little change as required in accordance with our laboratory condition. The 4th instar larvae of *Aedes aegypti* were taken as 5 groups with 20 numbers in each, and they were allowed to expose 249 ml. of water and 1.0 ml of examined solution bioenhancer (cow ark with *Plectranthus Amboinicus* plant extract concentration). An independent control was maintained separately without adding a test solution. After 24 hours exposure, the mortality rate was counted and means values were expressed, among 5 replicates of the experiments. Complete larval mortality was taken as criteria for carrying out further dose response analysis. Examined solution concentration was determined as 0.5 mg/ml 1 mg/ml, 4, 6, 8 and 10mg/ml. Control was maintained with 250 ml. distilled water. The glass container mouth was tightly closed with muslin cloth to prevent contamination. After 24 hours of exposure the effects were observed. Larvae death was confirmed by a mild touch with a glass rod to find their mobility or sedentary.

Statistical analysis

The obtained data base set was analyzed using probit analysis for determining LC 50, and LC 90 values, with 95% of confidence limit. An individual probit model was applied to

the examined each extract. Computation and statistical analysis were performed using SPSS software package.

3. RESULTS AND DISCUSSION

Phytochemical screening

In the present investigation, presence of phytochemical constituents in *Plectranthus Amboinicus* plant leaf extract combined with cow ark was analyzed. The obtained results were shown in the Table .1 Results deciphered that occurrence of common phytoconstituents, such as alkaloids, terpenoids, glycosides, steroids, flavonoids, phenolic compounds, Tannins, Quinones, Saponin, Droplets of fat and oil were detected (Table-1).

Table.1 Phytochemical screening of cow ark with leaf extract of *Plectranthus Amboinicus*

S.No	Tests	Detected in cow ark with plant extracts
1	Alkaloids	+
2	Glycosides	+
3	Terpenoids	-
4	Carbohydrates	+
5	Protein	+
6	Steroids	+
7	Flavonoids	+
8	Phenols	+
9	Tannins	-
10	Quinines	-
11	Saponins	-

Antimicrobial efficacy

The potential of the selected cow ark and leaf extract of *Plectranthus Amboinicus*, exhibited a significant antibacterial ability against all examined strains including two Gram positive and two Gram negative bacteria. Among them, an increased ability was detected, in antibacterial actions against Gram negative bacteria such as *P. aeruginosa* and *E. coli* rather than Gram positive strains observed. The Zone inhibition, an indicator of antibacterial activity, was found to be 15mm, 20mm, 18mm and 20mm against *B.Subtilis*, *P. Aeruginosa*, *S. Aureus* and *E. Coli* respectively at 75 Ug ml⁻¹ concentration (Table 2).

Table.2 Antibacterial activity of cow ark with plant extracts Herbal by agar well diffusion method

S.No	Micro organism	Concentration of extract (µl) /zone of inhibition (mm)			
		Control	25µl	50 µl	75 µl
1	<i>P. aeruginosa</i>	Nil	8	14	21
2	<i>E.coli</i>	Nil	6	12	18
3	<i>Bacillus subtilis</i>	Nil	5	13	16
4	<i>Staphylococcus aureus</i>	Nil	4	10	16

Mosquito Larvicide effects

In the present investigation, the potential or improved effectiveness of bio enhancer, cow ark with plant extract in larvicide effects on the Malaria causing mosquito, *Aedes aegypti* was examined. The mortality rate was recorded in accordance with varied concentration of test solution (Cow ark + *Plectranthus Amboinicus* extract) such as 0.5mg/mL, 2, 4, 6, 8 and 10 mg/mL, after aged for 24 hours exposure (Table-3).

Table.3 Larvicide activity of cow ark (bioenhancer with *Plectranthus Amboinicus*) against *A. aegypti*

Mosquito species	Concentration (µg/ml)	Mortality (%) ± SD	LC ₅₀ (µg/ml)	LC ₉₀ (µg/ml)
<i>Aedes aegypti</i>	25	33±1.53	47.661 µg/ml	99.329 µg/ml
	50	43±1.53		
	75	80±1.00		
	100	90±1.53		

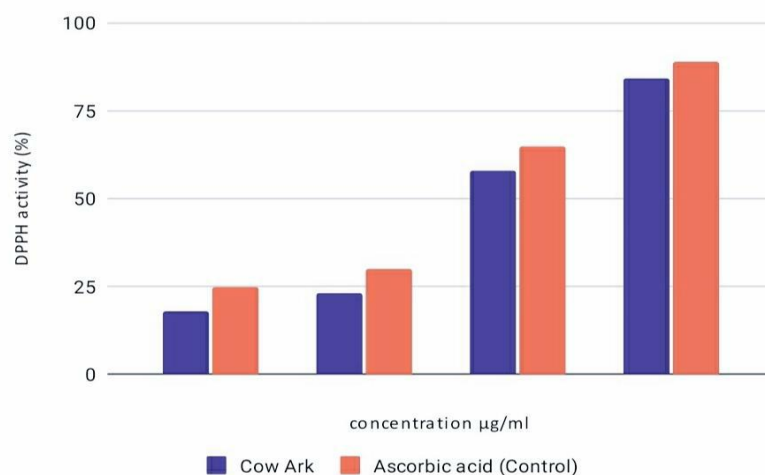
Average rate of mortality, and mean values of observed effects of examined solution was tabulated and compared with control, where in, exclusively, no test solution was added. Results exhibited that a profound mortality was detected, (90%) in the treatment group at 10mg/mL concentration whereas in control, the value was found to be 1.67% mortality. This finding proved its ability of larvicide against *A. aegypti* mosquito larvae.

Antioxidant potential

The capability of cow ark with plant leaf extract in scavenging the DPPH radical was measured. Results showed that the percentage of inhibition ability of the examined solution was found to be significant. Obtained values were expressed on the basis of dry weight (d.w.) and tabulated in (Fig- 1), Visual color change was noticed in the tested sample, as Green and Yellow color indicating to the respective cow ark and leaf extract exclusively. The antioxidant capacity executed in the investigation may be attributed to their phytochemical dominance.

Fig-1 DPPH antioxidant scavenging activity of Cow ark with *Plectranthus Amboinicus*

The graphical representation showed antioxidant potentials of cow ark(Bioenhancer), where Ascorbic acid was treated as control



A wide spectrum of advantages from indigenous cow ark has been receiving much attention and led to a thrust for understanding the chemical composition, as a bioenhancer. Several investigations suggested that cow urine is known to cure innumerable diseases to the human health concern. The pigs estrus urine having chief sources of pheromones and Pheromone compound for therapeutic potentials [11-13]. Findings of the present investigation reported that varied classes of Alkaloids, Flavonoids, Polyphenolic contents, Saponin, Tannin and droplets of Oils were observed. A study voiced that higher concentrations of phenolic, saponins and flavonoids were detected as the responsible factors to the diverse biological capability and antioxidant properties. Polyphenols from medicinal plant source is capable of arresting lipid peroxidation by exerting free radical scavenging phenomenon. It has been demonstrated that presence of glycosides in the examined enhancer, believed to be a profound contributor of antioxidant capacity, due to the possession of hydroxyl group [3]. Mothana et al. 2009 have reported that Saponin and Phenolic content present in the plant leaf extract might have been accountable for effective antioxidant properties in *Acacia* species was documented [14]. In the present investigation antioxidant ability of both cow ark and leaf extract have shown to be an effective antioxidant potential, which may be attributed to the occurrence of high specific antioxidant phytoconstituents, that helps to enhance the effectiveness of cow ark. The concentration values reported in the study was found to be close to the values of standard employed.

The metabolites involved in scavenging of an odd oxygen and free radical were directly associated with abundant biomaterials in the herbal extract. Therefore, cow ark and the leaf extract combination showed an excellent antioxidant property and promising potential to evaluate the biological action of biomolecules present in cow ark. According to Wate et al., 2011 cow ark has been effectively fluctuating and enhancing activity of

antibiotic, antifungal and anti-cancer therapeutic agents and served as a catalyst for bio active molecules [15]. In general, cow urine, traditionally used as disinfectant, purifier and proved to be excellent antiseptic compared with chemical based, presently accessible in the market. Despite numerous antibiotic and antimicrobial drugs commercially displayed, there is a lacuna in effectiveness against emerging threats or micro-organisms, resulting in a need of developing novel antibiotics against dreadful pathogens.

In the present investigation, tested bio enhancer, exhibited a remarkable anti-microbial activity against Gram negative bacterial pathogens such as *P. aeruginosa* and *E. coli* and inhibited at high limit at 75 U_g m⁻¹. Although leaf extract of *Plectranthus Amboinicus* showed beneficial properties, cow ark has been obtained from Indian cows and was proven to be highly effective in anti-microbial properties while its combination [2-3]. Several studies have demonstrated that cow urine with extract of *Azadirachta indica* exhibited a potential advantage against multi-drug resistance treatment against *E.Coli* and *K. pneumonia* was reported[16]. Hence, in the present findings, antimicrobial activity against two clinical pathogens exhibited maximum growth suppression, similar to the findings reported in early.

Further, potential of cow ark with leaf extract revealed a pronounced effects on the larvicidal activity against the 4th instant larvae of *A. aegypti* was assessed and reported. The obtained value, such as , concentration of 10 U_g/mL was an indicator of candidate activity of larvicide and may occur in the biomolecules of cow ark. Our findings are agreed in the line of earlier reports demonstrated by several investigators. Furthermore, there have been many effective drugs for antimalarial, such as, quinine, CLQ, and other classes of drugs compounds are in use, specifically plant based, and animal origin template compounds for the formulation are affordable [17-21]. Enormous accounts of studies have been showed that free radicals implicates against disorders covering diabetes cancer, antidiarrheal, antiinflammatory and wound healing[22-26]. The earlier studies reported that cow ark with *Acalypha indica*, *Tamarindus indica*, *Gymnema sylvestre* and *Murraya koenigii* have highly potential of antioxidant and antimicrobial against pathogenic bacteria[27-31].

This clearly indicated that chemical constituents present in cow ark , played a role effectively independently and when coupled with plant based materials, cow ark has been improving its effectiveness to achieve the therapeutic target, and served as a bioenhancer with a dominant potential to activate its properties.

4. CONCLUSION

The present investigation has obviously elucidated that findings observed are claimed to be a concrete evidence for enhancing capability of Cow ark, in antioxidant ,antimicrobial, biofilm inhibition and antimalarial potentials with a plant based materials to achieve and enhance extreme effectiveness of cow ark biomolecules, as an alternate therapeutic implications.

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