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PHYTOCHEMICAL EVALUATION AND BACTERICIDAL POTENTIAL OF *TERMINALIA ARJUNA* STEM BARK

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ABSTRACT

Antibacterial activity of methanolic extract of *Terminalia arjuna* stem bark samples (Apical bark, middle bark and Mature inner bark) was studied with respect to different pathogenic bacteria species *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoneae, Salmonella typhii*, *Proteus mirabilis* and *Micrococcus sp.* The methanolic extract of apical bark was effective than the middle bark and mature bark in inhibiting the growth of all bacteria. The bacterium *Staphylococcus aureus* was most sensitive among all the bacterial species studied. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, flavanols, phenols, saponins and terpenoids. The concentrations secondary metabolites was found higher in the apical stem bark than the middle and mature stem bark. The percent extract yield was maximum in apical stem bark.

INTRODUCTION: The pathogenic microbial infectious agents developed chemotherapeutic and antibiotic resistance has lead to screening of several medicinal plants for their potential antimicrobial activity [1]. Higher plants as a source of medicinal compounds plays a dominant role in human health [2]. Various studies have been carried out to extract the various natural products for screening antimicrobial activity [3, 4, 5]. Plant based anti- microbials have enormous therapeutic potential. They are effective in the treatment of infectious disease, simultaneously mitigating many of the side effects that are associated with the synthetic antimicrobials. Medicinal plants represent a rich source of antimicrobial agent [6]. It has been estimated by WHO that 80% of the people living in the developing countries rely upon the traditional health practices for their primary health care needs [7].

Terminalia arjuna belongs to the family combretaceae. *T. arjuna* is distributed throughout India, Burma and Sri Lanka and mainly grows along the banks of the river and streams [8]. Arjuna is a known potent cardiotonic since ancient times [9]. Bark powder boiled with water and is inhaled to cure headache and to kill worms in the teeth [10]. According to Kumar and Jain [11], people living in the South Surguja district of Madhya Pradesh uses the bark of *T. arjuna* in the treatment of fever and high blood pressure. People living in the Malkangiri district of Orissa, chew the fresh bark of *Terminalia arjuna* and the juice is used as antacid [12]. Decoction of the bark is used as ulcer wash, while bark ash is used in the treatment of the snakebite and scorpion sting [9]. Paste of bark in water is applied externally on the site of fractures and helps in the early healing. Taking into account of this wide use bark of *Terminalia arjuna*, present study is aimed to evaluate the antimicrobial potential of its bark samples against the different human pathogenic bacteria.

MATERIALS AND METHODS:

Collection and processing of the plant material: Different bark samples (Apical bark, middle bark and mature inner bark) of *Terminalia arjuna* were collected from the hilly regions of Kolhapur district. The bark was collected in the month of May 2009. The bark samples were cut into pieces, sun-dried then oven dried at 60^{o}C. Dried bark samples were ground into powder and stored in an air tight plastic container.

Microorganisms: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsciella pneumoneae, Salmonella typhii*, *Proteus mirabilis and Micrococcus sp.* were used for testing antibacterial activity of bark extracts. The test organisms used in this study were obtained from the department of Microbiology, Shivaji University, Kolahapur, Maharashtra, India. The bacterial strains were cultured on nutrient agar slants. The cultures were maintained by subculturing periodically and preserved at 4^{o}C until further use.

Preparation of the extract: Oven dried 10g of powdered bark material was weighed accurately and placed in soxhlet extraction chamber which was suspended above the flask containing 100mL of 80% methanol and below a condenser. The flask was heated and the methanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded at certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and methanol was evaporated by using rotary evaporator. The weight of the residual extract was measured and percent yield was calculated. The residue of the extract was dissolved in 25ml of pure methanol and stored in air tight glass vials at 4^{0}C until further use [13].

$$\text{Extract yield \%} = \frac{W1}{W2} \times 100$$

Where; W1= Net wt of powder in grams after extraction; W2= total wt of wood powder in grams taken for extraction.

Qualitative screening of phytochemicals: Different extracts were screened for the presence of alkaloids, glycosides, flavonoids, flavanols, phenols, saponins and terpenoids by using standard protocols [15, 16].

Preparation of the media: Accurately weighed 28g of nutrient agar (Himedia) was dissolved in the 1000ml of distilled water. The medium was sterilized under 15lb pressure for 15 minutes in an autoclave. 30ml of this sterilized semisolid nutrient agar medium was poured in pre-sterilized 90mm glass petriplates under aseptic conditions in laminar flow. The plates were allowed to cool at room temperature to solidify the medium.

Determination of antibacterial activity by agar well diffusion method: Agar well diffusion method was employed to determine antibacterial activity [14]. Well of 10mm diameter was prepared with sterilized cork-borer. Standard antibiotic Chloramphenicol at 50µg/ml was served as positive control and Methanol as negative control. The plates inoculated with different bacterial species were incubated at 37^{o}C in incubator for 24h and the zone of inhibition was measured (Diameter in mm). All of the experiments were performed in triplicate. The results are reported as the average of 3 experiments.

RESULTS: Preliminary phytochemical screening (**Table 1**) of methanolic extract of bark revealed the presence of some secondary metabolite alkaloids, glycosides, flavonoids, flavanols, phenols, saponins and terpenoids.

TABLE 3: ANTIBACTERIAL ACTIVITY OF STEM BARK OF *TERMINALIA ARJUNA*

	Minimum inhibitory concentration Zone of inhibition (Diameter in 'mm')*							
Microorganisms	**Bark sample**	**25µL**	**50 µL**	**100 µL**	**200 µL**	**300 µL**	**M**	**C (50µg/ml)**
Bacillus subtilis	1	0.00	1.70 ± 0.43	3.63 ± 0.50	5. 67 ± 0.00	8.67 ± 0.52	0.00	21.50 ± 1.05
	2	0.00	1.00 ± 0.57	3.37 ± 0.52	5. 00± 0.57	7.50 ± 0.55	0.00	
	3	0.00	0.00	1.50 ± 0.51	3.50 ± 0.55	6.67 ± 0.52	0.00	
Staphylococcus aureus	1	0.00	2.39 ± 0.21	5.50 ± 0.22	8.33 ± 0.42	10.00 ± 0.63	0.00	11.17 ± 0.98
	2	0.00	1.76 ± 0.26	5.5 ± 0.72	8.00 ± 0.51	10.50 ±0.67	0.00	
	3	0.00	1.41 ± 0.42	5.33 ± 0.45	8.83 ± 0.45	10.33 ± 0.53	0.00	
Escherichia coli	1	0.00	1.57 ± 0.11	3.33 ± 0.52	6.67 ± 0.58	8.67 ± 0.62	0.00	16.50 ± 0.55
	2	0.00	1.33 ± 0.19	3.17 ± 0.41	6.00 ± 0.63	8.33 ± 0.63	0.00	
	3	0.00	1.11 ± 0.23	3.17 ± 0.41	5.50 ± 0.84	8.00 ± 0.52	0.00	
Pseudomonas aeruginosa	1	0.00	2.00 ± 0.30	6.33 ± 0.52	8.67 ± 0.82	11.00 ± 0.63	0.00	14.33 ± 0.5
	2	0.00	1.87 ± 0.17	4.67 ± 0.82	8.00 ± 0.61	10.33 ±0.77	0.00	
	3	0.00	1.12 ± 0.88	4.50 ± 0.55	7.83 ± 0.73	11.00 ± 0.63	0.00	
Salmonella typhii	1	0.00	2.10 ± 0.19	4.83 ± 0.75	6.83 ± 0.52	10.67 ± 0.82	0.00	16.67 ± 1.21
	2	0.00	1.53 ± 0.00	3.83 ± 0.75	6.33 ± 0.41	9.33 ± 0.82	0.00	
	3	0.00	1.13 ± 0.31	3.17 ± 0.75	6.17 ± 0.77	9.17 ± 0.69	0.00	
Klebsiella pneumoneae	1	0.00	2.58 ± 0.43	3.87 ± 0.02	4.53 ± 0.88	7.29 ± 0.31	0.00	14.85± 1.17
	2	0.00	1.76 ± 0.27	2.70 ± 0.21	3.17 ± 0.65	5.83 ± 0.38	0.00	
	3	0.00	1.60 ± 0.69	1.97 ± 0.36	2.24 ± 0.18	4.73 ± 0.53	0.00	
Proteus mirabilis	1	0.00	1.89 ± 0.25	2.31 ± 0.67	5.41 ± 0.91	8.19 ± 0.29	0.00	13.70 ±0.49
	2	0.00	1.53 ± 0.36	1.88 ± 0.74	3.69 ± 0.15	6.33 ± 0.13	0.00	
	3	0.00	1.02 ± 0.63	1.37 ± 0.41	2.47 ± 0.61	5.77 ± 0.86	0.00	

Micrococcus sp	1	0.00	2.17 ± 0.55	3.89 ± 0.75	5.82 ± 0.17	7.93 ± 0.59	0.00	16.63 ± 0.23
	2	0.00	1.70 ± 0.69	2.44 ± 0.35	4.73 ± 0.10	6.66 ± 0.26	0.00	
	3	0.00	1.00 ± 0.23	2.29 ± 0.76	4.00 ± 0.07	5.49 ± 0.58	0.00	

1: Apical Bark, 2: Middle Bark and 3: Mature inner Bark; C: Chloramphenicol M: Methanol; *: Agar well diffusion method; Values are mean ± SD of three replicates

The concentration of phytochemicals was found higher in the apical stem bark than the middle bark and mature bark. The apical stem bark shown higher percent extract yield than the middle and mature bark on main trunk (**Table 2**).

TABLE 2: PERCENT EXTRACT YIELD

Plant name	Bark sample	Summer
Terminalia arjuna	Apical bark	26.11%
	Middle bark	25.87%
	Mature inner bark	24.29%

Antibacterial activity of stem bark extract of *Terminalia arjuna* against different bacterial pathogens is presented in the **Table 3**. From Table 3 it is clear that all the bacterial pathogens were inhibited at minimum inhibitory concentration of 50µL of all the bark extracts (Apical, middle and mature bark extracts.). The inhibitory effect was increased with dose dependant manner and at a maximum concentration of 300µL all bacteria were highly inhibited. The apical bark showed maximum inhibitory effect than the middle and mature bacteria.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF BARK OF *TERMINALIA ARJUNA*

Sample	Phenols	Flavones	Flavonoids	Tannin	Terpenoids	Saponin	Alkaloids	Cardiac glycosides
1	+++	+++	+++	+++	+++	+++	+++	+++
2	++	++	++	++	++	++	++	++
3	+	+	+	+	+	+	+	+

1-Apical bark; 2-Middle bark and 3- Mature inner bark; +++: Present in high concentration, ++: Present in moderate concentration and +: Present in low concentration

At maximum concentration of 300µL, the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* were highly inhibited and showed maximum inhibition zones in the range 10mm and 11mm respectively. The bacteria *Escherichia coli* and *Salmonella typhii* showed inhibition zones in the range of 8mm and 9.50mm respectively while the inhibition zone bacterium *Bacillus subtilis* was in the range of 7mm. The three bacteria namely *Klebsiella pneumoneae, Proteus mirabilis* and *Micrococcus sp* were found resistant with minimum inhibition zone in the range 5.5mm.

DISCUSSION: Flavonoids, tannins, alkaloid, and other secondary metabolite serve as defense mechanism against predations by many micro-organisms, insect and herbivores [17, 18]. Tamokou *et al*., [19] isolated xanthones, physcion, friedelin and friedelanol, of these, xanthones and physcion exhibited the antimicrobial activities against bacteria *S. typhii*, *K. pneumonae*, *P, aeruginosa*, and *B. subtilis* and four yeast species *Candida albicans*, Candida *tropicalis*, *Candida parapsilosis* and *Cryptococcus neoformans* respectively. Many xanthons and anthraquinons have shown this type of activity [20, 21]. Eight flavonoids betulin, betulinic acid, oleanolic acid, quercetin, (-) epicatechin, gallic acid, and β-systosterol isolated from methanol fraction of *Brysoniama crassifolia*. These compounds were found active against many bacteria including *S. aureus*, *B. subtilis*, *K. pneumonae* and *P. aureginosa* [22]. The bark extract was found to be containing tannin glycosides, alkaloids, steroids and Flavonoids which are biologically active [23]. Phenolics and polyphenols present in the plants are known to be toxic to the microorganism [24]. Tannin from *Dichrostachys cinerea* root bark possesses antibacterial activities

against *S. aureus*, *E. coli* and *P. aeruginosa* [25]. Flavonoids have been reported to have both antibacterial and antifungal activities [26]. In our study all the three bark samples revealed the presence of secondary metabolite. The high inhibitory potential of methanolic extract might be due to the high solubility of the phytoconstituents in the polar organic solvent like methanol. Presence of these phytoconstituents in the stem bark points towards the pharmacological activities of *Terminalia arjuna* and supports the claim of the traditional users.

CONCLUSION: The reasons for high inhibitory potential by the apical stem bark might be due to the high secondary metabolite content. Another fact for difference in the bacterial potential among the apical, middle and mature stem bark that the active principle present in the apical bark might be absent in the mature bark and if present may not be active against the bacterial species. Again, one compound active against few bacteria may not inhibit the growth of the other bacterial species and hence reflecting the differential antibacterial potential among the three bark sample. The high inhibitory effect exhibited by apical stem bark might be due to presence or synthesis of some new active phytochemical. Thus, it is of prime important to know the biochemistry of apical bark in order to screen the new pharmacological active principles.

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