

Therapeutic efficacy of *Cinnamomum tamala* (Buch.-Ham.) and *Aegle marmelos* (L.) leaf

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ABSTRACT: Medicinal plants are widely used for therapy of various diseases and disorders due to presence of various bioactive phytochemicals. Among the studied phytochemicals polyphenol content was higher (16.7 ± 0.7 g/100g in *C. tamala* and 6.7 ± 0.61 g/100g in *A. marmelos*) and flavonoids content was lower in both plants (1.0 ± 1.01 g/100g and 0.9 ± 0.25 g/100g in *C. tamala* and *A. marmelos* respectively). Leaf extract of both plant were effective against *S. aureus* and *P. mirabilis* (6-10 mm ZOI and 100% inhibition at 0.25mg-5mg of leaf extracts in agar disk diffusion and broth dilution method respectively) and the leaf extract of both plant possess high antioxidant activity, 0.9% and 0.2% at 5 μ g/mL concentration 4% and 7% at 100 μ g/mL concentration of *A. marmelos* and *C. tamala* leaf extracts respectively. Aqueous leaf extract of both the plants did not show cytotoxicity by haemolysis at 0.2 mg/mL-1mg/mL concentration.

Key -words: Phytochemical, disease, pathogenic, antioxidant, haemolytic.

Introduction

Infectious diseases are caused by pathogenic microorganisms such as *Staphylococcus aureus* and *Proteus mirabilis* are and are transmitted from one host to another via vectors [1]. *S. aureus* causes infection of wounds; urinary tract and *P. mirabilis* cause urethritis, cystitis, pyelonephritis, prostatitis and pneumonia [2].

Free radicals ROS and NOS (Reactive oxygen species and Reactive Nitrogen Species) are constantly formed in the human body but their excessive production during diseases and tissue injuries causes oxidative stress by hampering nucleic acids, lipids, and proteins, which ultimately leads to cellular cell death [3] which in turns in leads to various disorders like, Parkinson's disease, Allergic encephalomyelitis, Ocular hemorrhage, Atherosclerosis, Keshan disease, Autoimmune nephrotic syndromes, endotoxin liver injury, diabetogenic actions of alloxan, aging and cancer [4,5].

Medicinal plants contain wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which

are associated with antioxidant and other medicinal properties against various disease and disorders [6] but plant saponins are associated with haemolysis and saponins are also used as adjuvant in vaccination [7].

Aegle marmelos commonly known as bael, belonging to the family rutaceae and *Cinnamomum tamala* belonging to family lauraceae have been tested against the pathogenic bacteria and .These plants are frequently used as natural antioxidant and medicine for treatment of various diseases and disorders such as wounds, urinary tract infection, urethritis, cystitis, pyelonephritis, prostatitis and pneumonia [8-10].

The present study is an attempt to evaluate the phytochemical screening, antipathogenic efficacy against *S. aureus* and *P. mirabilis*, total antioxidant capacity and cytotoxicity of leaf extract of *C. tamala* and *A. marmelos*.

Materials and methods

Collection of Plant material:

The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by

using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required [11].

Extract preparation:

50 g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ~350 mL methanol and distilled water separately. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies [11].

Phytochemical screening:

Total phenol was determined by Folin-Ciocalteu reagent, following Ramamoorthy and Bono [12]. Tannins were quantified as stated in the Quality control methods for medicinal plant materials [7]. Aluminium chloride colorimetric method was used with some modifications to determine flavonoids content [13]. Alkaloid was determined by the method used by Helrich [14]. Saponin content was determined following Obdona and Ochuko [15]. The details have been described elsewhere Kumar *et al.* [16, 17].

Anti-bacterial analysis:

Antibacterial efficacy of methanolic leaf extract of *C. tamala* and *A. marmelos* was carried out against *Proteus mirabilis* (MTCC 1249) and *Staphylococcus aureus* (MTCC 3160). In agar disk diffusion method comparing with standard antibiotic Gentamycin following Threlfall *et al.* [18] and by broth dilution method as proposed by Walker [19]. The details have been described elsewhere Kumae *et al.* [20].

Antioxidant assay:

Total antioxidant capacity of aqueous extract was determined spectrophotometrically and expressed as equivalent to ascorbic acid. Butylated hydroxy anisole (BHA) was used as reference standard [21]. The details have been described elsewhere Kumar *et al.* [22].

Cytotoxicity:

Hemolytic activity of the aqueous leaf extract was determined using goat blood. An erythrocyte suspension was prepared by adding 5% (by volume) of sodium citrate (36.5 g/L) to fresh blood and centrifuged at 1000 rpm for 5 min to separate the erythrocytes. 2% erythrocyte suspension was prepared by adding 49 mL phosphate buffer (pH 7.4) to 1 mL packed erythrocytes. Serial dilution of plant extracts were prepared using phosphate buffer. 1 ml of citrated blood was mixed with equal volume of diluted plant extracts and the volume was adjusted to 5 mL by phosphate buffer. The mixture was allowed to stand for 20 minutes at room temperature and O. D. was measured spectrophotometrically at 540 nm [7, 23].

Results and discussion

Phytochemical screening:

The results of phytochemical analysis of the leaf samples of *C. tamala* and *A. marmelos* are presented in Figure - 1. The result revealed that polyphenols is highest (16.7 ± 0.7 g/100g and 6.7 ± 0.61 g/100g in *C. tamala* and *A. marmelos* respectively) and flavonoids occur in lowest quantity (1.0 ± 1.01 g/100g and 0.9 ± 0.25 g/100g in *C. tamala* and *A. marmelos* respectively) among all the studied phytochemicals. Kumar *et al.* [16], reported 6.13 ± 0.13 g/100g tannin, 2.09 ± 0.17 g/100g saponin, 2.1 ± 0.21 g/100g flavonoids, 0.13 ± 0.1 g/100g poly phenols in *A. vasica*. Dandapat *et al.* [10] also reported 1.38 ± 0.5 g/100g tannin, 4.5 ± 0.63 g/100g saponin, 0.65 ± 0.2 g/100g flavonoid, 1.73 ± 0.4 g/100g polyphenol and 2.6 ± 0.5 g/100g alkaloid in *T. cordifolia*. Phytochemical composition of *A. marmelos* and *C. tamala* have found in higher concentration than most of the studied plants. Tannin acts as an antibacterial substance by destabilization of cytoplasmic and plasma membranes, inhibition of extracellular microbial enzymes and metabolisms, and deprivation of the substrate required for microbial growth [25] or by inactivate microbial adhesions,

enzymes, cell envelope transport proteins, and mineral uptake due to presence of hydroxyl (-OH) groups which possess toxicity to microorganisms [26]. The tannins also exert a direct protective effect against oxidative stress-induced cell death [27]. Saponins inhibit microbial growth by inhibiting DNA dependent RNA synthesis in saponine treated host [28] and are directly associated with antioxidant activities on serum lipids [29]. Flavonoids prevent cellular injury by direct scavenging of free radicals and inhibit carcinogenesis [30] and

suppress bacterial growth by inhibiting action of several enzymes, chelate certain metal cations, affect protein phosphorylation [31]. Alkaloids possess anti-oxidizing effects, thus reduce the nitrate generation which is useful for protein synthesis, suppresses the transfer of sucrose from stomach to small intestine and also possess antibacterial activity [32]. Dandapat *et al.* [6] reported plant phenolics are potent inhibitors of a number of growth factor binding and signalling pathways implicated in cancer.

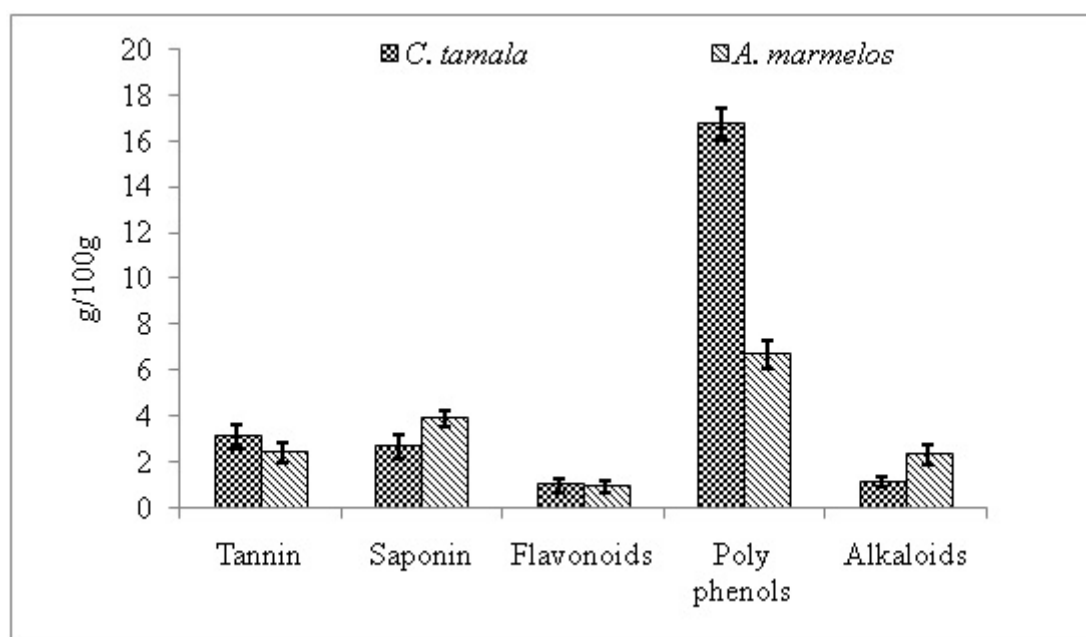


Figure 1: Phytochemicals from *C. tamala* and *A. marmelos* leaf in g/100g ($M \pm SD$; $n = 3$)

Antibacterial assay:

The anti pathogenic efficacy of methanolic extract of *C. tamala* and *A. marmelos* leaves were quantitatively assessed comparing with gentamycin on the basis of zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) following agar

diffusion method (in Table-1 and 2) and by broth dilution method (Fig-2 and 3). In the present investigation extract of the both plants were found to be effective against both the pathogens (Table-1) with compared standard antibiotic gentamycin (Table-2) in agar diffusion method.

Table 1: ZOI and MIC of *C. tamala* and *A. marmelos* against *S. aureus* and *P. mirabilis*

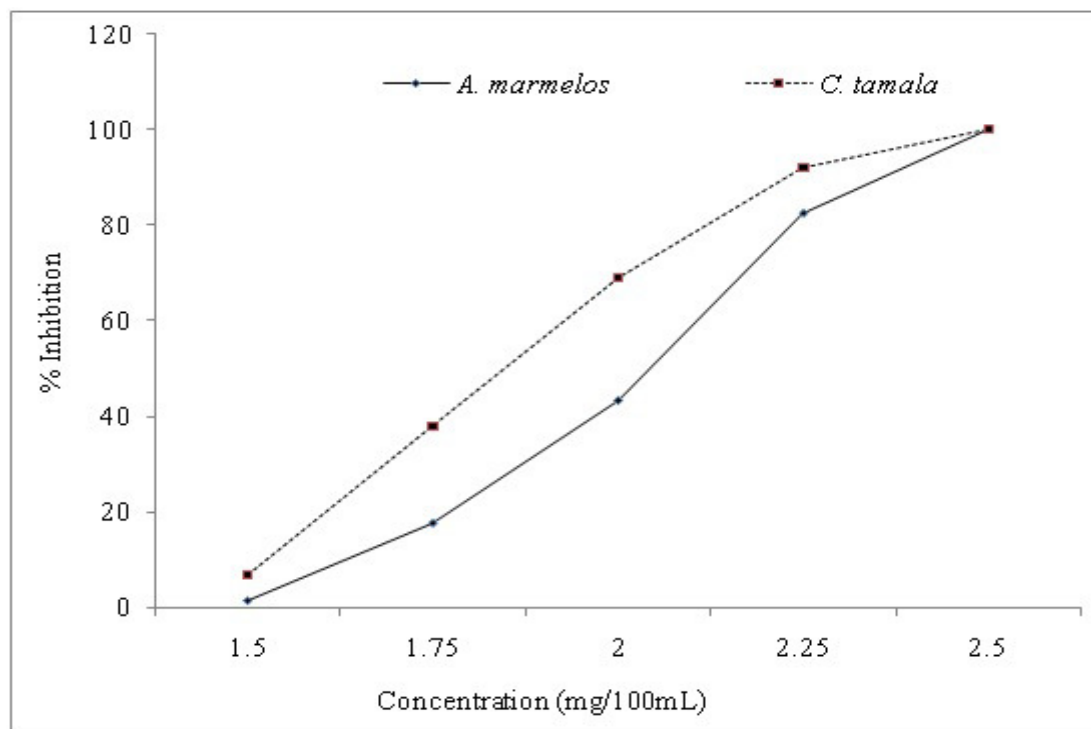
Concentration (mg/mL)	Zone of inhibition (in mm)			
	<i>C. tamala</i>		<i>A. marmelos</i>	
	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>S. aureus</i>	<i>P. mirabilis</i>
0.18	0	0	0	0
0.36	0	0	0	0
0.612	0	0	0	0
1.25	0	0	0	6
2.5	6	0	4	9
5	6	10	5	10
MIC (mg/mL)	2.5	5	2.5	1.25

Table 2: ZOI and MIC of Gentamycin against *S. aureus* and *P. mirabilis*.

Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm) against gentamycin	
	<i>S. aureus</i>	<i>P. mirabilis</i>
25	13	9
50	18	13
100	21	18
200	25	21
400	27	25
800	34	27
MIC ($\mu\text{g/mL}$)	25	25

The broth dilution method showed more pronounced antimicrobial activity extract of the both plants through 100% inhibition of

both the pathogens in the range of 1.25mg-5mg/mL concentration.

**Figure 2: % Inhibition of *S. aureus* in broth dilution method for methanolic leaf extract of *C. tamala* and *A. marmelos*.**

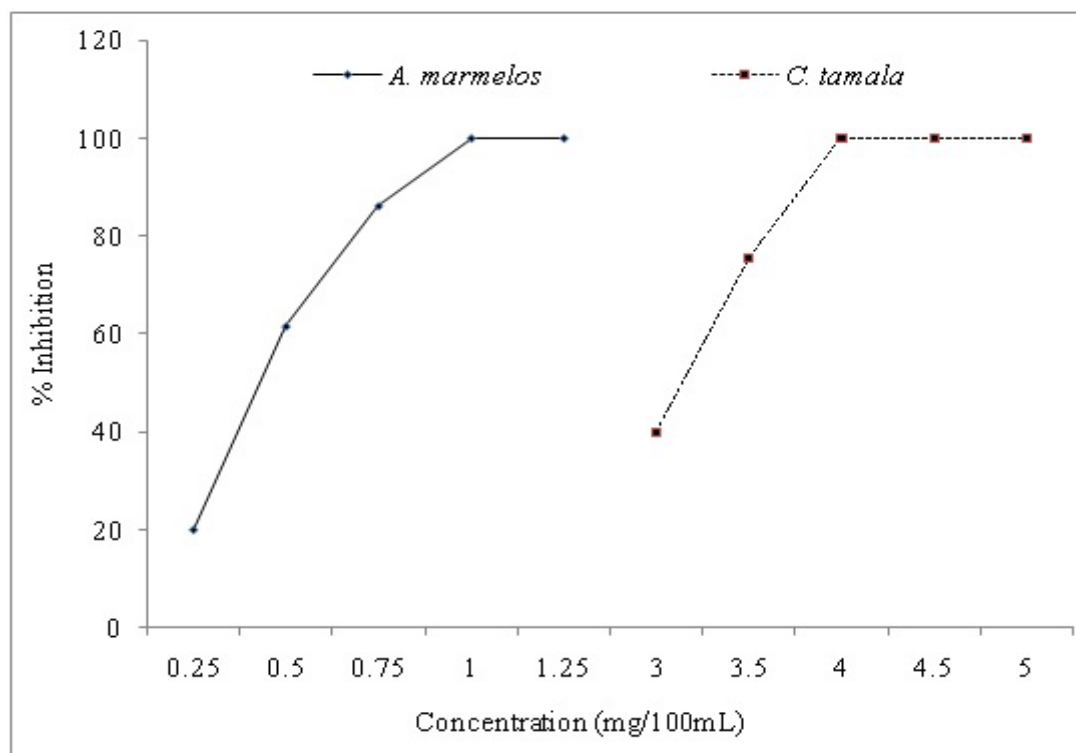


Figure 3: % Inhibition of *P. Mirabilis* in broth dilution method for methanolic leaf extract of *C. tamala* and *A. marmelos*.

Kumar *et al.* [7,4] worked out on methanolic extract of *Calotropis procera* and found 4mm-10mm ZOI in agar diffusion method. They also reported the plant extract showed 100% inhibition at 10mg/mL and 5 mg/mL concentration against *S. aureus* and *P. miabilis* respectively and also said that methanolic extract is more effective than other extracts because polar and non polar components of the plant material are effectively extracted through an organic solvent. *C. tamala* possess antibacterial activity due to the presence of different phenolic compound such as cinnamaldehyde, eugenol and cinnamic acid etc. An important characteristic of these components is hydrophobicity, which enable them to partition the lipids of the bacterial

cell membrane and mitochondria, disrupt the cell structure and rendering the more permeable [33]. Extensive leakages from bacterial cells or exits of critical molecules and ions will lead to death [34]. The antibacterial activity *A. marmelos* leaf extract is due to presence of active phenolic compound eugenol and cuminaldehyde they inhibit protein synthesis either at transcription or at translation level and also inhibit synthesis of peptido-glycan [35].

Antioxidant assay:

Result of total antioxidant activity of aqueous extract of both the plants expressed as the mg/mL of ascorbic acid /100mg presented in figure-3. *C. tamala* showed higher antioxidant activity (7%) equivalent to ascorbic at 100 μ L of leaf extract.

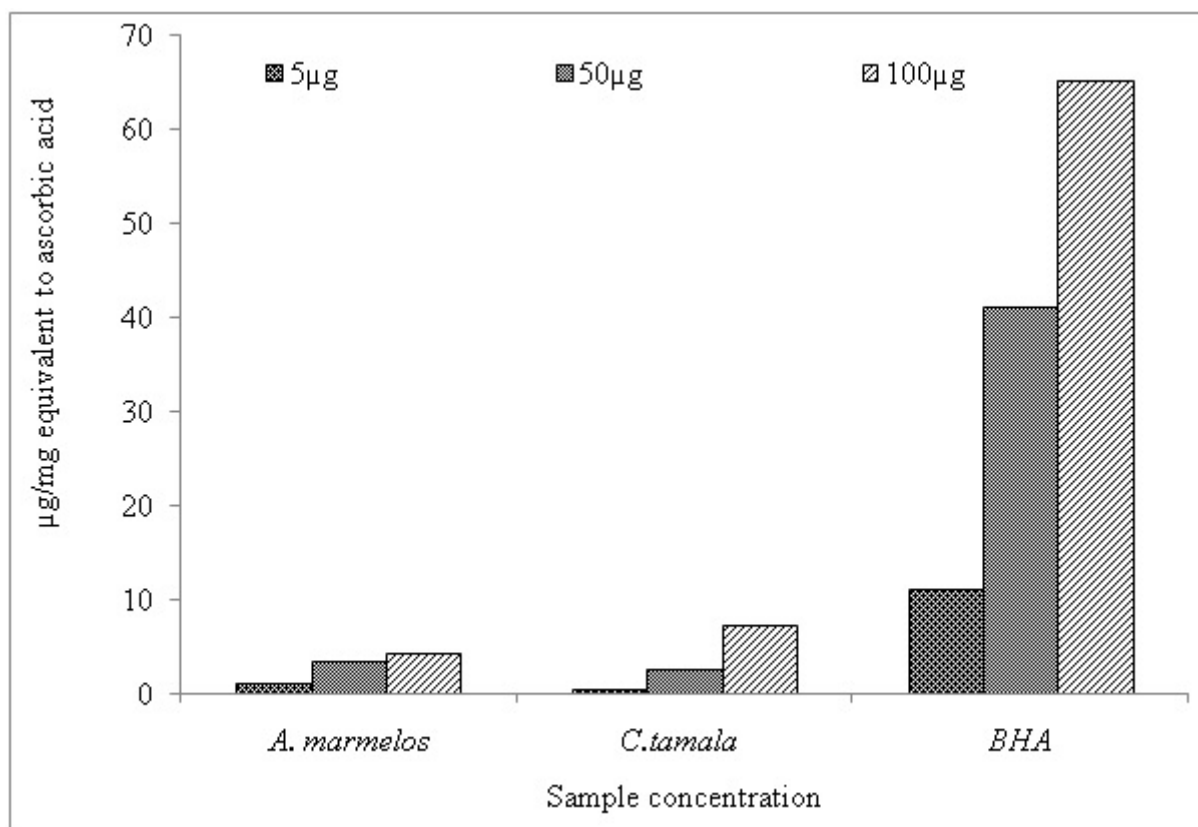


Figure 4: Total antioxidant activity of *A. marmelos* and *C. tamala* leaf extract in comparison with Butylated hydroxyl anisole (BHA).

Kumar *et al.* [5] reported 0.1%, 4%, 8% and 0.2%, 0.8%, 2.3% total antioxidant activity of *V. negundo* and *A. vasica* respectively. Kumar *et al.* [17] reported total antioxidant activity of *C. procera* is 0.1%, 0.2% and 0.3%. *C. tamala* and *A. marmelos* possess high antioxidant activity among the studied plants. *A. marmelos* contains bioactive compounds like eugenol and marmesinin are independently showed their activity in oxidative stress by free radical scavenging [36]. *C. tamala* also possess eugenol and another bioactive chemical linalool have free radical

scavenging capacity due to presence of electron repelling group in *o*- position [37].

Cytotoxicity:

Result of haemolytic activity of *C. tamala* and *A. marmelos* presented in fig-5 and 6. Leaf extract of both the plant did not show haemolysis at 0.2 mg/mL – 1 mg/mL. Kumar *et al.* [23] reported 0.2 mg/mL - 0.4mg/mL leaf extract of *C. procera* is safe but more concentrated leaf extract increase in the hemolytic activity with time of incubation. Since *C. tamala* and *A. marmelo* did not show haemolysis, hence the plant extract is non cytotoxic.

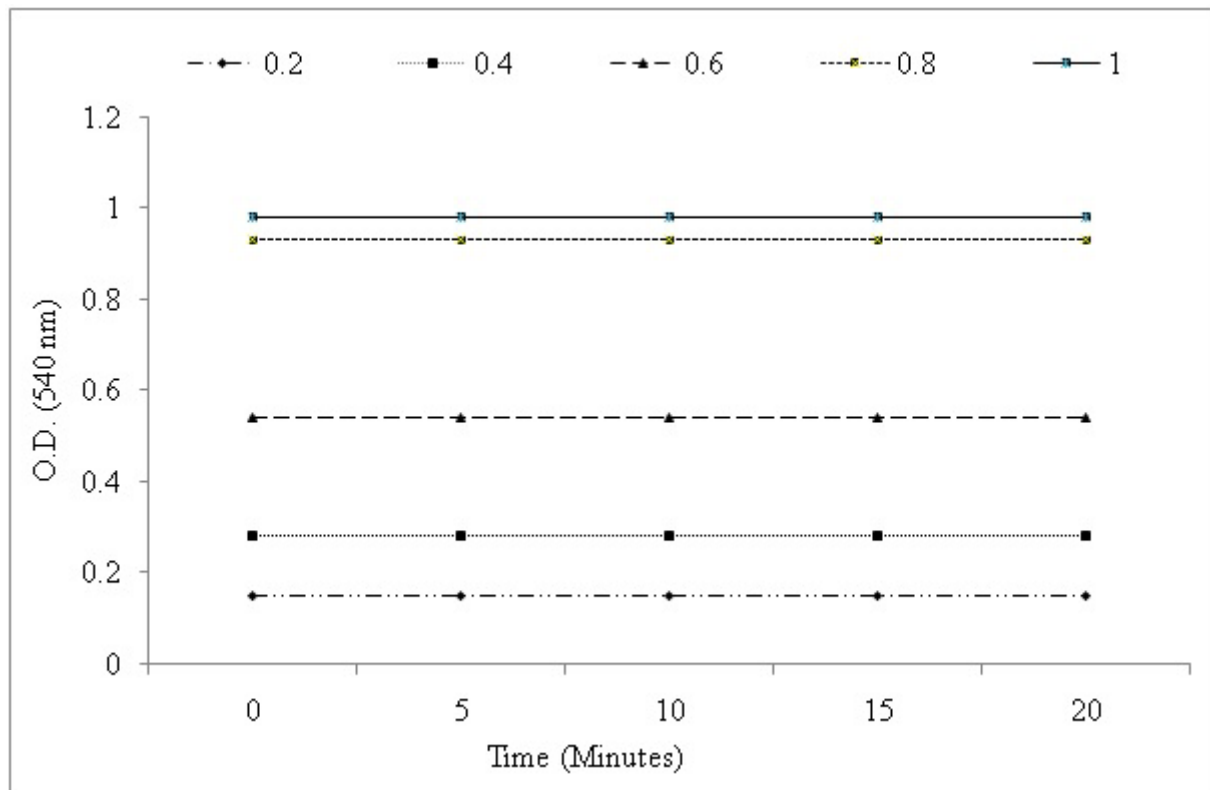


Figure 5: Cytotoxicity of *A. marmelos* leaf extract by Haemolytic method.

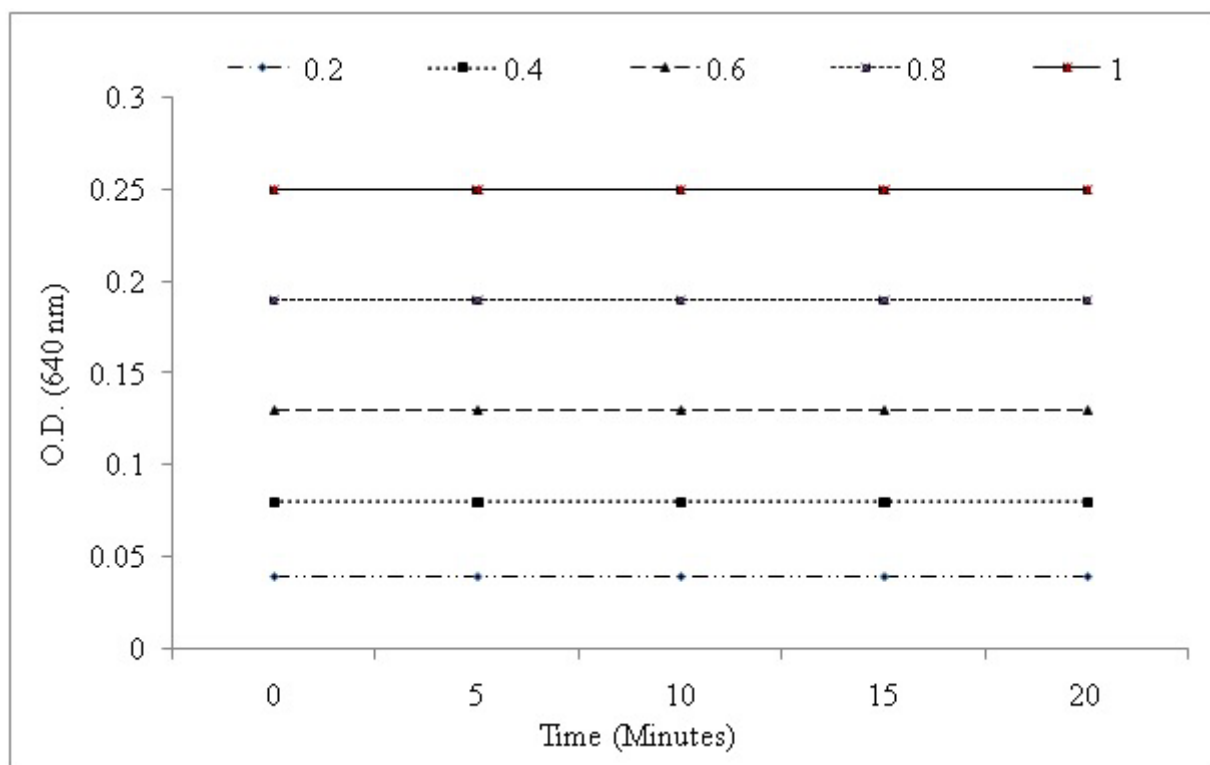


Figure 6: Cytotoxicity of *C. tamala* leaf extract by Haemolytic method.

4. Conclusion:

In spite of presence of phytochemicals in *C. tamala* and *A. marmelos*, possess high antioxidant and anti-pathogenic activity, the leaf extracts of above plants is safe at higher concentration, thus it can be used as medicinal supplement.

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