

## PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF *KYDIA CALYCINA* ROXB., AERIAL PARTS

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### Abstracts

**Back ground:** Herbal remedies used in traditional medicine contain a wide range of substances which are used to treat acute and chronic infectious diseases. The development of drug resistance in human pathogens against normally used antibiotics has necessitated a search for new antimicrobial agents from other natural sources mostly plants. This study was undertaken to investigate the in vitro response of the extracts and soluble fractions of *Kydia calycina* Roxb., aerial parts against eight pathogenic bacterial strains

**Method:** Using agar cup plate method the hydro alcoholic (methanol 80%) extract, hexane, ethyl acetate and methanol soluble fractions of *Kydia calycina* Roxb. aerial parts were subjected to antibacterial activity at the concentrations of 20,40, 80 and 160mg/ml. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide (DMSO) which were used as a vehicle alongside the standard drug Rifampicine.

**Result:** From the results it was observed that the hydro alcoholic (methanol 80%) extract of *Kydia calycina* exhibited significant antibacterial activity at the concentrations tested. At the concentrations of 25 and 50mg/ml, the hexane and ethyl acetate soluble fractions showed significant inhibitory effect against *Basillus megaterium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus vulgaris* and moderately against *Streptococcus pneumonia*, *Bacillus subtilis* and *E. coli*.

**Conclusion:** The demonstration of antimicrobial activity against the bacteria strains is an indication that the plants are potential sources for production of drugs with a broad spectrum of activity. The results of the study also support the traditional applications of the plants and suggest that the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents.

**Keywords:** *Kydia calycina* Roxb., antibacterial activity, hydro-alcoholic extract, hexane, ethyl acetate and methanol soluble fractions, inhibition zone, phytoconstituents

### 1. Introduction

Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens are considered as potential source for different classes of antimicrobial substances<sup>1</sup>. Plants used in traditional medicine contain a wide range of substances which are used to treat chronic as well as acute infectious diseases. The substances either inhibit the growth of micro-organisms or kill them are considered for developing new drugs for treatment of various infectious diseases<sup>2</sup>. The development of drug resistance in human pathogens against normally used antibiotics has necessitated a search for new antimicrobial agents from other sources primarily plants. Herbal medicines have been used in developing countries as an alternative to allopathic medicines because they are safe, cost

effective and show very minimal or no side effects compared to synthetic drugs.

Traditional knowledge of ethnic people is always a great source for discovery of new drugs. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development. Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques<sup>3</sup>. The phytoconstituents such as phenols, anthraquinones, alkaloids, glycosides,

flavonoids and saponins are antibiotic principles of plants. From these phytoconstituents, saponins have anti-inflammatory been reported to exhibit hemolytic and foaming activity, antifungal, antibacterial, anti-inflammatory, fungi static, molluscidal activity<sup>4</sup>.

*Kydia calycina* Roxb. (Synonyms - *Kydia fraterna* Roxb. and *Kydia roxburghiana* Wight). is native of Asia-tropical, India, Indo-china Pakistan, Nepal, Bhutan and Myanmar. The plant is a deciduous tree, growing up to 10- 20m tall; Leaves broad, ovoid to nearly orbicular, 3-5 angular or lobed, dark green and less hairy above, grayish below. Flowers: white or pink, axillary or terminal panicles with prominent epicalyx segments. Fruit- globose capsule, seeds reniform, hairy. Plants are mucilaginous, anti inflammatory, febrifuge; leaf<sup>5</sup> and root are anti rheumatic, paste of the leaves applied for body pains and leaves are used in poultices for skin diseases. The plant extracts are used in treating liver disorders and skin related problems<sup>6</sup>. *Kydia calycina* is being used by the local people and tribal folk of north coastal districts of Andhra Pradesh for liver ailments.

With this back ground of the plant's medicinal values the present study was undertaken to investigate the in vitro response of the extracts and soluble fractions of the plant *Kydia calycina* Roxb., aerial parts.

## 2. Materials and methods

### 2.1 Collection of the plant material:

The aerial parts of *Kydia calycina* were collected from the forests of Eastern Ghats, Paderu, Visakhapatnam district, Andhra Pradesh, South India (Voucher no. VPJ/DOB/KC1409)

### 2.2 Extraction procedure

Freshly collected plant materials were shade dried, coarsely powdered in WIELLY mill and exhaustively extracted with 80% methanol in Soxhlet apparatus for 48 hours. The extracts were concentrated to dryness in Rota vapor till free from the solvents. The mass obtained was weighed and the percentage yield was calculated. Sufficient quantity of extract was kept aside for biological activity. The extracts of *Kydia calycina* aerial parts were suspended separately in little water and fractionated with hexane, Ethyl acetate and methanol in order of polarity to get three fractions, hexane soluble, ethyl acetate soluble and finally the remaining is methanol soluble fraction. The alcohol insoluble portion was kept aside. All fractions were

distilled to remove solvent and finally concentrated under reduced pressure and controlled temperature (50-60 °C) to get their corresponding residues.

### 2.3 Qualitative Phytochemical Screening

A spectrum of natural compounds like triterpenoids, alkaloids, glycosides, tannins, flavonoids, essential oils and other similar secondary metabolites which exert physiological activity are synthesized in the plant, in addition to the carbohydrates, proteins and lipids utilized by main source of food materials.

A systematic and complete study of crude drugs should include a thorough investigation of both primary and secondary metabolites derived as result of plant metabolism. Different qualitative chemical tests are performed for establishing profile of a given extract/fraction for its nature of chemical composition<sup>7-10</sup>.

In the present study, methanol extracts and their hexane, ethyl acetate, methanol soluble fractions of *Kydia calycina* aerial parts were subjected to qualitative chemical tests using standard procedure to detect various phytoconstituents present in them.

### 2.4 Determination of zone of inhibition by cup plate method<sup>11</sup>:

The cup plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds.

A sterile borer was used to prepare cups of 6 mm diameter in the agar medium spread with the selected micro-organisms at the rate of 0.1 ml of inoculum in 15ml of sterile molten agar<sup>12</sup>. Accurately measured (0.05 ml) solution of each concentration test extract/fraction/compounds of the selected plants and reference standards were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to 8 °C for a period of 2 hours for effective diffusion of test extracts/fractions/ compounds and standards. Later, they were incubated at 37 °C for 24 hours for bacterial cultures and 25 °C for 48 hours in case of fungal cultures. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity.

The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide (DMSO) which were used as a vehicle alongside the standard drug Rifampicine.

The test organisms are *Streptococcus pneumonia*, *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*. The experiments were performed in triplicates. The diameter of the zone of inhibition was measured and recorded. The antimicrobial activity of the extracts and soluble fractions was determined by comparing their inhibition zones to that of standard drug.

### 2.5 Statistical Analysis

The diameter of zones of inhibition were measured and the results (mean value n=3) were recorded by measuring the zone of growth inhibition around the cups. Values are the mean of three values  $\pm$ SEM.

## 3. Results

### a) Preliminary phytochemical screening

Qualitative phytochemical screening of hydro alcoholic extract of *Kydia calycina* aerial parts revealed the presence of steroids, triterpenoids, alkaloids, flavonoids, tannins and glycosides. Its hexane soluble fraction showed phytosterols, triterpenoids, alkaloids and glycosides. Ethyl soluble fraction revealed the presence of steroids, triterpenoids, alkaloids, flavonoids, tannins and glycosides. Whereas the methanolic soluble fraction showed the presence of triterpenoids, flavonoids, tannins and glycosides (Table: 1).

### b) Antibacterial activity

With the increase in resistance of microorganisms to the currently used antibiotics, limited antimicrobial spectrum and the increased side effects of synthetic drugs, efforts to find out

a broad spectrum based, inexpensive and alternate health care system with less side effects have kindled interest and promoted studies on traditionally claimed medicinal plants<sup>12,13</sup>. Many plants have been found to possess antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant eg. The components with phenolic structures were highly active against the microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents depending upon the concentration used<sup>12</sup>.

The hydro alcoholic (methanol 80%) extract of *Kydia calycina* exhibited significant antibacterial activity at the concentrations tested (20, 40, 80 and 160mg/ml). Hence the hexane, ethyl acetate and methanol soluble fractions were also tested at the same concentrations. From the results it was observed that hexane and ethyl acetate fraction showed prominent activity, whereas methanol soluble fraction showed moderate antibacterial activity. At the concentrations of 20 and 40mg/ml, the hexane and ethyl acetate soluble fractions showed significant inhibitory effect against *Bacillus megaterium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus.vulgaris* moderately against *Streptococcus pneumonia*, *Bacillus subtilis* and *E. coli*. Whereas at the concentration of 40mg/ml, methanol soluble fraction showed significant activity against *Streptococcus pneumonia*, *Bacillus megaterium*, *Pseudomonas aeruginosa* and *Proteus.vulgaris* when compared with the standard drug (Table: 2; Figure 1).

**Table 1: Nature of phytoconstituents present in different extracts of *Kydia calycina***

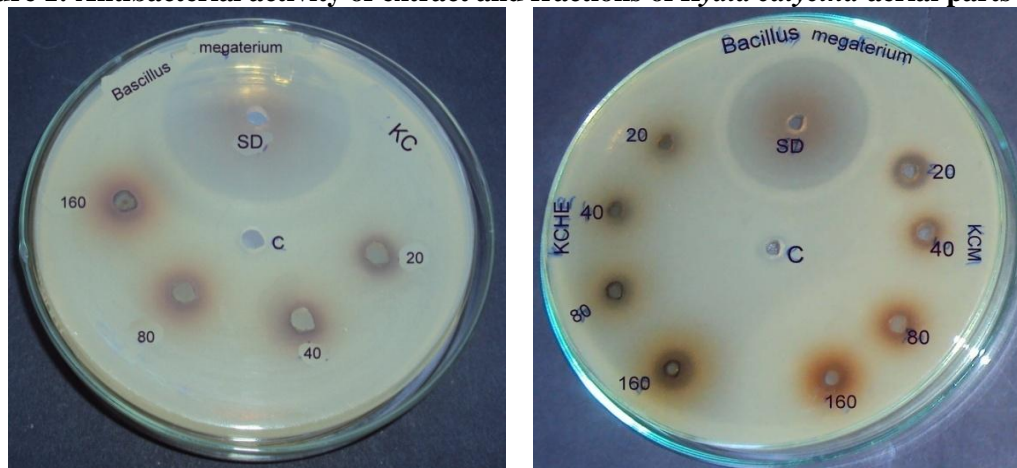
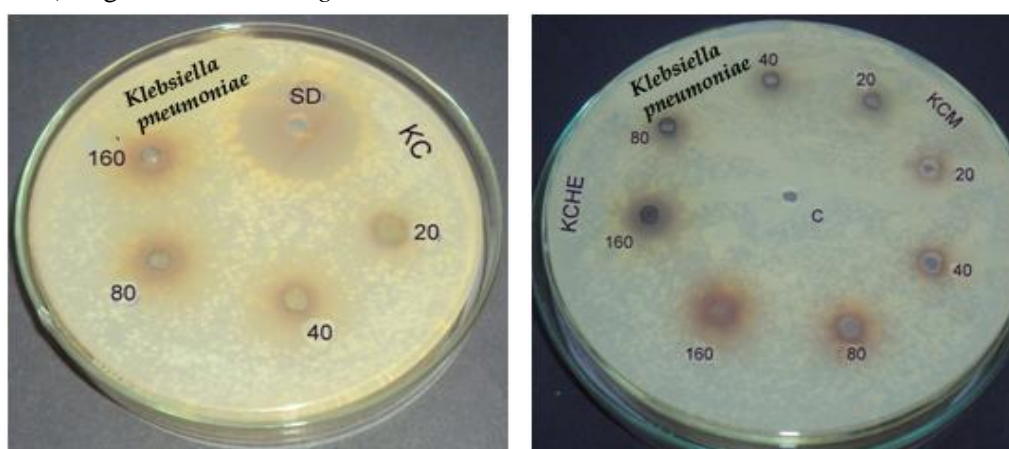
Phytoconstituent	Hydro alcohol extracts (80%)	Hexane soluble fraction	Ethyl acetate soluble fraction	Methanol soluble fraction
Phytosterols	+	+	+	+
Triterpenes	+	+	+	+
Saponins	-	-	-	-
Alkaloids	+	+	+	-
Flavonoids	+	-	+	+
Tannins	+	-	+	+
Carbohydrates	-	-	-	-
Glycosides	+	+	+	+

+ = Present, - = absent

Table 2. Anti-bacterial activity of extract and fractions of *Kydia calycina*

Test extract/fraction of <i>K. calycina</i>	Dose (mg/ml)	Zone of inhibition <sup>#</sup> ( mm)							
		Gram +ve bacteria				Gram -ve bacteria			
		<i>S.p.</i>	<i>B.s</i>	<i>B.m</i>	<i>S.a</i>	<i>E.c</i>	<i>K.p</i>	<i>P.a</i>	<i>P.v</i>
Hexane fraction	20	08 ±0.40	08 ±0.52	10 ±0.26	12 ±0.34	08 ±0.55	14 ±0.33	10 ±0.52	11 ±0.18
	40	10 ±0.22	10.5 ±0.24	11.5 ±0.16	13 ±0.33	10 ±0.22	15 ±0.31	11.5 ±0.42	12.5 ±0.23
	80	12 ±0.18	12 ±0.26	12 ±0.35	13.5 ±0.33	12 ±0.15	17.5 ±0.12	14 ±0.26	13 ±0.21
	160	14 ±0.64	14 ±0.14	14 ±0.27	15 ±0.55	13 ±0.32	20 ±0.12	18 ±0.33	14 ±0.21
Ethyl acetate Fraction	20	10 ±0.62	09 ±0.45	9.5 ±0.13	11 ±0.05	09 ±0.60	13 ±0.71	10 ±0.25	10 ±0.14
	40	12 ±0.21	11 ±0.50	11 ±0.37	12.5 ±0.48	11 ±0.20	14 ±0.22	12 ±0.43	12 ±0.24
	80	13 ±0.18	13 ±0.30	12.5 ±0.09	13 ±0.14	12.5 ±0.34	16 ±0.51	15 ±0.16	14 ±0.24
	160	15 ±0.53	14.5 ±0.71	15 ±0.33	16 ±0.48	14 ±0.35	18 ±0.42	18.5 ±0.65	14.5 ±0.12
Methanol Fraction	20	12 ±0.27	08 ±0.22	12 ±0.08	10 ±0.55	12 ±0.76	15 ±0.32	09 ±0.44	08 ±0.15
	40	15 ±0.08	10 ±0.11	13 ±0.9	12 ±0.48	14 ±0.52	16 ±0.33	11.5 ±0.14	10 ±0.21
	80	16 ±0.24	10 ±0.44	14 ±0.23	12.5 ±0.32	--	--	13 ±0.66	10 ±0.51
	160	18 ±0.45	11 ±0.33	15 ±0.48	13 ±0.60	--	--	--	11.5 ±0.21
Hydro alcoholic Extract (80% Methanol)	20	08 ±0.73	08 ±0.34	10 ±0.45	09 ±0.22	08 ±0.17	10 ±0.20	10 ±0.31	10 ±0.43
	40	11 ±0.26	11 ±0.16	10.5 ±0.48	10 ±0.59	10 ±0.64	11.5 ±0.18	12 ±0.37	11 ±0.42
	80	13 ±0.19	--	12 ±0.20	11.5 ±0.66	12 ±0.49	15 ±0.15	13 ±0.44	11.5 ±0.35
	160	14 ±0.42	--	13 ±0.21	13 ±0.33	13.5 ±0.15	16 ±0.54	15 ±0.86	12.5 ±0.39
<b>Standard Rifampicine</b>	5	30 ±0.43	21 ±0.50	35 ±0.40	24 ±0.31	32 ±0.60	28 ±0.11	40 ±0.27	25 ±0.9
<b>Vehicle- DMSO</b>	-	-	-	-	-	-	-	-	-

*S.p* = *Streptococcus pneumoniae*; *B.c* = *Bacillus subtilis*; *B m* = *Bacillus megaterium* ; *S.a* = *Staphylococcus aureus*; *E.c* = *Escherichia coli*; *K. p* = *Klebsiella pneumonia*; *P.a* = *Pseudomonas aeruginosa*; *P.v* = *Proteus vulgaris* #Values are the average of triplicate; Includes the cup diameter (6mm)

**Figure 1: Antibacterial activity of extract and fractions of *Kydia calycina* aerial parts**A) Against *Bacillus megaterium*B) Against *Klebsiella pneumoniae*

KC: *Kydia calycina* hydro alcoholic extract; KCHE: *Kydia calycina* hexane fraction extract; KCM: *Kydia calycina* methanol fraction extract; SD: Standard Drug, Rifampicine; C: Control

#### 4. Discussion

The present study on qualitative phytochemical screening isolation and structure elucidation of the bioactive phytoconstituents from the methanol extract of *Kydia calycina* aerial parts confirmed the presence of sterols, alkaloids, flavonoids, acids, saponin glycosides, terpenoids, tannins, proteins and carbohydrates, fixed oil in general, squalin,  $\beta$ -sitosterol, stigmasterol and a flavonoid glucoside, tiliroside in specific. Literature review shows that Phytochemical studies on the Cyclopropenoid and fatty acid composition of *Kydia calycina* seed oil showed the presence of lauric, myristic, palmitic, stearic, arachidic, behenic, oleic, linoleic acids and cyclopropenoid fatty acid<sup>14</sup> and a sesquiterpenoid naphthol was reported from *Kydia calycina* Roxb.<sup>15</sup> The leaves of *Kydia calycina* were reported to be used for anti-rheumatism<sup>5</sup>. The methanol extract of leaves of *Kydia calycina* Roxb. was screened for analgesic and anti-inflammatory activity<sup>16</sup>.

Literature review revealed that the compounds  $\beta$ -sitosterol<sup>17</sup>, Kaempferol derivatives, vitexin and isovitexin<sup>18</sup> showed antimicrobial activity. As the extract tested positive for saponins and tannins, these polar compounds may also contribute to the antibacterial activity<sup>19-22</sup> of the hexane, ethyl acetate and methanol soluble fraction of the plant. The presence of both these polar and non polar antimicrobial compounds may contribute to the significant antibacterial activity of the methanol extract and the fractions of *Kydia calycina* aerial parts.

#### 5. Conclusion

The demonstration of antimicrobial activity against both Gram – negative, Gram-positive bacteria strains is an indication that the plants are potential sources for production of drugs with a broad spectrum of activity. The results of the study also support the traditional applications of the plants and suggest that the

plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents.

Antimicrobial assays on plant extracts/fractions are valuable in screening the presence of antimicrobial activity. However, such assays do not provide true quantitative measure of the activities of some components present in the extract / fraction such as the polar and large molecules which have poor mobility in the water-agar medium<sup>19</sup>. Further testing of extracts and isolated compounds against a broad spectrum of micro organisms needs to be carried out to establish their potential as source of antibiotics against human pathogens.

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