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Identification of bioactive compounds and cytotoxic activity of *Careya arborea* Roxb. leaves

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Abstract

Careya arborea Roxb. commonly known as wild guava, widely available in India and has a variety of traditional uses. The present study was undertaken to explore the cytotoxic potential of *Careya arborea* leaves. Successive extracts of selected for qualitatively and quantitatively estimated for phytochemical. The polyphenol rich extracts were evaluated for cytotoxic potential using Human nasopharynx (KB), human lung (HOP62), human cervix (ME180) and human leukemia (K562) cancerous cell lines. The ethanol extract of *Careya arborea* demonstrated most potent anticancer activity through its lowest GI₅₀, TGI (amount for 50% Growth Inhibition and Total Growth Inhibition) concentration. Furthermore, the chromatographic study of ethanol extract showed the presence of gallic acid, chlorogenic acid, rutin and quercetin as polyphenols. The antioxidant mechanism of these polyphenols may be responsible for cytotoxic activity. The present study confirmed anticancer effect of *Careya arborea* leaves and could be a potential source for future anticancer drugs.

Keywords: Cytotoxic, *Careya arborea* leaves, polyphenol, anticancer

Introduction

Cancer has become one of the most annihilating diseases globally with more than 10 million new cases annually. In the discovery of anticancer drugs, plants have been played significant role, as over 60% of anticancer agent are derived from natural sources^[1]. India is also one of rich source for anticancer and immunomodulatory plants^[2]. Several plants consist of ethno medicinal claims for their cytotoxic and immunomodulatory potential^[3]. Many ethnobotanically recommended and traditionally used medicinal plants might contain chemical substances with potential mutagenic and/or carcinogenic properties as well as with antitumor properties^[4]. There is always a need to search alternative sources with more effectiveness, more accessibility, and least side effects. Therefore, the present study was carried out to explore the anticancer activity of *Careya arborea* Roxb. leaves extracts in cultured Human nasopharynx (KB), human lung (HOP62), human cervix (ME180) and human leukemia (K562) cell lines.

Careya arborea Roxb. commonly known as wild guava, is a medium-sized deciduous tree; exhibiting dark grey colour and exfoliating in thin strips. It is widely available in India, Ceylon, Malay and Peninsula. The plant has a variety of traditional uses. The leaves are used for orally in fever while applied locally to relieve swelling^[5]. The juice of the leaves is applied in ulcers and skin diseases in India^[6]. Leaves found to contain triterpenoids and steroids such as taraxerol, n-hexacosanol, α -spinasterol, taraxerol, taraxeryl acetate, 2 α hydroxy ursolic acid, Triterpene ester-careaborin and β -sitosterol. It also reported to contain tannins and flavonoids and tannins as sitosterol, ellagic acid and quercetin^[7-9]. The bio-activity guided fractionation of methanol extract of leaves reported to present triterpenoids saponines with good antileishmanial activity. Previous works have shown that *C. arborea* bark and leaves possess antimicrobial activity^[10].

Material and Methods

Collection and extraction

Leaves of *C. arborea* were collected at Mahur-Kinwat region of Nanded district, Maharashtra. The plant was identified by Prof. Vishal R. Marathe, Science College, Nanded, and herbarium voucher specimen was deposited.

The air-dried leaves of *C. arborea* were powdered and exhaustively extracted with different polarity solvent, successively. The petroleum ether (60-80^o) (PE-CA), chloroform (CH-CA), ethyl acetate (EA-CA), and ethanol (EO-CA) extracts were filtered, evaporated under reduced pressure to obtain a viscous dried extracts.

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Phytochemical screening

The *C. arborea* leaves extracts were preliminary investigated for presence of secondary metabolites using standard qualitative chemical tests described by Harborne [11] for detection of phytochemicals.

Total Phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method [12]. 100 µl of test extracts were mixed thoroughly with 2 ml of 15% Na₂CO₃. After 2 min to this mixture, 200 µl of Folin-Ciocalteu reagent was added and incubated at room temperature for 30 min. The absorbance was measured at 760 nm against a blank. The standard calibration curve was prepared using gallic acid in place of test extract. Total phenolic content was expressed milligram of gallic acid equivalents (GAE) per gram of dried extract. It was calculated by using regression equation.

Total Flavonoids content

The different concentrations (20-100 µg/ml) of standard quercetin solutions (0.5 ml) were separately mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminium nitrate, 0.1 ml of 1 M sodium acetate and 2.8 ml of water. The resultant mixture was kept at ambient temperature for 40 min. The absorbance of reaction mixture was measured at 415 nm; calibration curve was plotted for concentration against absorbance. Same procedure was followed for the extracts. In the blank solution, the volume of 10% aluminium nitrate was substituted with the same volume of distilled water [13]. The total flavonoid content in the extract expressed as milligram per gram of quercetin equivalents (QE) with formulae as mentioned for total phenolic content.

HPLC analysis of extract

The presence of polyphenols bioactive extracts were estimated qualitatively by HPLC, as per the method described by Pawar and Surana [14]. The HPLC analysis was carried out for the identification of gallic acid, chlorogenic acid, catechin, rutin and quercetin in EO-CA using standard phytochemical marker. A Shimadzu HPLC system with LC-10AT, UV detector (Spectra System UV1000), and Luna C18 reverse-phase column (250 mm x 4.6 mm, i.d. particle size 5µ) was used. The isocratic mode with Methanol: Water (50:50) pH-3.0 with ortho-phosphoric acid with 0.7 ml/min flow rate at column temperature was 25°C and UV detection at 280 nm

Anticancer activity by SRB assay

On the results of qualitative and quantitative phytochemical estimation the EA-CA and EO-CA were considered as bioactive extracts and further proceeds for anticancer potential using SRB assay.

Anticancer activity of *C. arborea* extracts by sulforhodamine B assay was evaluated against 4 human cancerous cell lines at Cancer Research Institute of Tata Memorial Centre, Mumbai, India according to the reported method [15]. They were Human nasopharynx (KB), Human lung (HOP62), Human cervix (ME180), Human leukemia (K562) cancer cell lines. In SRB assay, successive PE-CA, CH-CA, EA-CA and EO-CA extract were tested. Cancer cells were plated (5x10³ cells

well-1) for 24 h before treatment with test samples to allow the attachment of cells to the plate. All the extracts were dissolved in DMSO and different concentrations of these test extracts were added to the cells monolayer. Triplicate wells were repeated for each dose and the cells incubated for 48 h at 37°C with 5% CO₂, then the cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with 10 mM trizma base and the absorbance was read on a micro plate reader at a wavelength of 540 nm with 690 nm as reference wavelength. LC₅₀, TGI and GI₅₀ was calculated in comparison to Doxorubicin as reference standard (Pharmacia Ltd-India).

Results and discussion

Screening of herbal drugs may lead to discovery of new mutagenic agents which can be an alternative source to the costly anticancer chemotherapeutic agents. Due to low toxicity and less cost, some medicinal plants have attracted the attention as alternative cancer therapies traditionally used since ancient times and have been of much interest due to historical claims of anticancer properties. This is the first report describing the scientific evidence and support to the claims laid by the folklore use of *C. arborea* leaves for its anticancer potential.

The percent extractive yield of successive *C. arborea* leaves extracts were found as 1.45 %, 1.90%, 2.30%, and 2.60% for PE-CA, CH-CA, EA-CA and extract, respectively. Among the successive extracts, the EO-CA shown the highest extractive value (2.60%); while PE-CA shown least extractives (2.30%). The successive extraction scheme for medicinal plant extraction used most widely as separates the phytochemicals based on their polarities and used biological evaluation. The high EO-CA extractive values of plants indicated, selected plant contains sufficient amount of secondary metabolites. In addition, amongst the all extracts preliminary phytochemical analysis the EO-CA (Table 1) shown most potent antioxidant components such as phenolics, flavonoids and saponins [16].

Table 1: Proximate chemical analysis of *C. arborea* leaves successive extract

Tests	PE-CA	CH-CA	EA-CA	EO-CA
Alkaloid	-	+	+	-
Glycoside	-	-	-	-
Saponins	-	-	+	+
Steroid	+	+	+	-
Triterpenoids	+	+	+	-
Tannins	-	+	+	+
Flavonoid	-	+	+	+
Test for carbohydrates	-	-	+/-	+
Test for proteins	-	-	-	-
Test for fats and lipids	+	+	-	-

(-): absent, (+): present; PE-CA - *C. arborea* petroleum ether (60-80°) extract; CH-CA - *C. arborea* chloroform extract, EA-CA: *C. arborea* ethyl acetate extract, EO-CA: *C. arborea* ethanol extract

The quantitative chemical analysis were validates the preliminary chemical analysis. The EO-CA contains highest amount of total phenolics, while EA-CA shown to contains total flavonoids (Table 2).

Table 2: Total phenolic and flavonoid content of *C. arborea* leaves extracts

Extracts	Total phenolic (mg/g gallic acid)	Total flavonoid (mg/g quercetin)
PE- CAL	3.64 ± 0.91	3.33 ± 0.82
CH-CAL	16.16 ± 1.57	6.67 ± 0.82
EA- CAL	26.36 ± 2.40	33.33 ± 2.18
EO- CAL	33.03 ± 1.39	25.71 ± 1.43

(n=3) mean ± SD; PE-CA - *C. arborea* petroleum ether (60-80°) extract; CH-CA – *C. arborea* chloroform extract, EA-CA: *C. arborea* ethyl acetate extract, EO-CA: *C. arborea* ethanol extract

The qualitative HPLC analysis of EO-CA extract showed the presence of gallic acid, chlorogenic acid, rutin and quercetin (Figure 1).

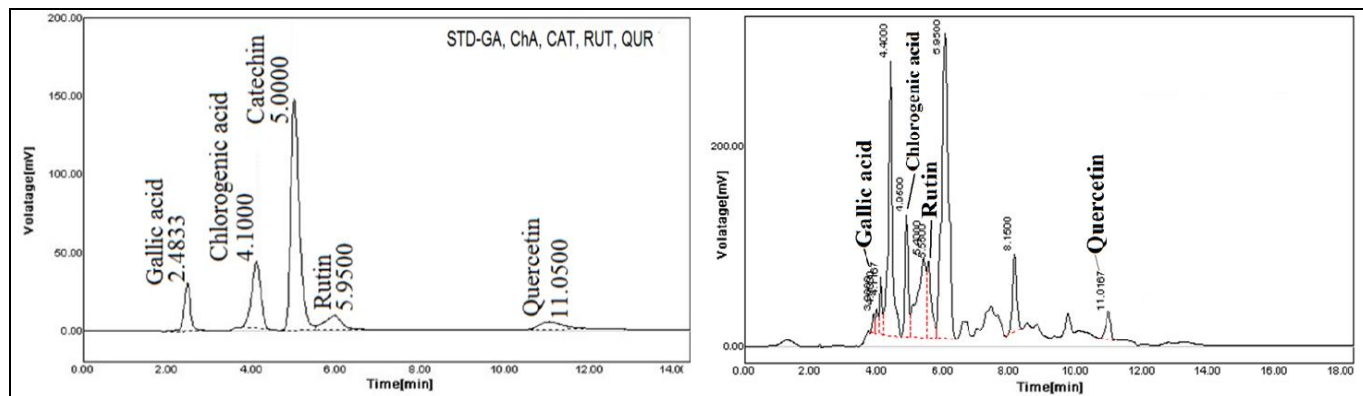


Fig 1: Chromatogram of standard marker quercetin, rutin, gallic acid, chlorogenic acid, catechin and ethyl acetate extract of *C. arborea*.

The anticancer activity of successive extracts of *C. arborea* leaves extracts on four selected human cell lines was assayed using SRB assay and all test sample showed cytotoxic effect. The EO-CA was found to be most potent cytotoxic extract when compared to EA-CA, as TGI and GI₅₀ was found to be less than 80 µg/ml in human nasopharynx (KB), leukemia (K562), cervix (ME180) and lung (HOP62) cancerous cell line. The lowest concentration of TGI and GI₅₀ were found in

ascending order in studied cancerous cell lines as Hop62 > ME180 > KB=K562.all the extract shown concentration-dependent growth inhibition of human cancerous cell-lines. Both the studied extracts, EA-CA, EO-CA found effective against ME180 cell lines (Table 3). Present anticancer study on human cancerous cell lines demonstrated that EO-CA compound was most active as compared to EA-CA.

Table 3: Anticancer activity of *C. arborea* leaves extracts on human cancer cell lines

Drug/extract	Human Nasopharyngeal cancer cell line (KB)			Human lung cancer cell line (Hop62)			Human cervix cancer cell line (ME180)			Human leukaemia cell line (K562)		
	LC ₅₀	TGI	GI ₅₀	LC ₅₀	TGI	GI ₅₀	LC ₅₀	TGI	GI ₅₀	LC ₅₀	TGI	GI ₅₀
EA-CA	> 80	> 80	> 80	> 80	> 80	53.6	> 80	73.5	66.3	> 80	> 80	58.4
EO-CA	> 80	72.6	66.3	> 80	56.5	43.4	> 80	58.6	45.3	> 80	74.6	64.4
Standard (Doxorubicin)	44.6	8.2	<10	79.2	40.2	<10	51.9	16.2	<10	49.2	12.2	<10

EA-CA: *C. arborea* ethyl acetate extract, EO-CA: *C. arborea* ethanol extract LC₅₀: 50% Lethal concentration, TGI: Total growth inhibition, GI₅₀: 50% growth inhibition.

This indicates that, the moderate polar constituents i.e. polyphenolic substance of plants were found to be more effective anticancer agents. The presence quercetin, rutin, catechin, gallic acid and chlorogenic acid in bioactive extract confirmed by HPLC analysis. The polyphenols influence apoptotic signaling pathways to bring about cell death by activating proapoptotic proteins and inhibiting anti-apoptotic proteins action^[17].

Conclusion

This study revealed the presence of phenolics and flavonoids in the leaf ethyl acetate and ethanol extracts of *C. arborea*. The plant exhibited promising antiproliferative activities against four human cancer cell lines may be due to its free radical scavenging activities and reducing potential, due to presence of bioactive polyphenols i.e. quercetin, rutin, catechin, gallic acid and chlorogenic acid. Thus, the results of the present study revealed the cytotoxic potential *C. arborea*.

Reference

- Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981– 2002. *Journal of natural products*, 2003; 66:1022-1037.
- Balekar N, Bodhankar S, Jain D. Screening Methods for Immunomodulatory Agents-A Review. *Indian drugs*, 2006; 43:525.
- Pezzuto JM. Plant-derived anticancer agents. *Biochemical pharmacology*. 1997; 53:121-133.
- Lombardi VR, Carrera I, Cacabelos R. *In vitro* screening for cytotoxic activity of herbal extracts. *Evidence-Based Complementary and Alternative Medicine*, 2017.
- Maheshwari J, Kalakoti B, Lal B. *Ethnomedicine of Bhil tribe of Jhabua District, MP. Ancient Science of life*, 1986; 5:255.
- Das P, Misra M. Some ethnomedicinal plants of Koraput district Orissa. *Ancient Science of life*, 1988, 8:60.

7. Mahato S, Banerjee S, Chakravarti R. Taraxerol from *Careya arborea*. Bulletin of the Calcutta School of Tropical Medicine, 1967; 15:8.
8. Gupt R, Chakraborty N, Dutta T. Crystalline constituents from *Careya arborea* Roxb. Indian Journal Pharmacy, 1975; 376:161-162.
9. Talapatra B, Basak A, Talapatra S. Terpenoids and related compounds. Part XX. Careaborin, a new triterpene ester from the leaves of *Careya arborea*. Journal of Indian Chemical Society, 1981; 58:814-815.
10. Kumar RS, Sivakumar T, Sundaram RS, Sivakumar P, Nethaji R, Gupta M. Antimicrobial and antioxidant activities of *Careya arborea* Roxb. Stem bark. Iranian Journal of Pharmacology & Therapeutics, 2006; 5:35-41.
11. Harborne A: Phytochemical methods a guide to modern techniques of plant analysis. Springer science & business media, 1998.
12. Singleton V, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 1965; 16:144-158.
13. Kalaskar MG, Surana SJ. Free radical scavenging and hepatoprotective potential of *Ficus microcarpa* L. fil. bark extracts. Journal of natural medicines, 2011; 65:633-640.
14. Pawar C, Surana S. Estimation of gallic acid, epicatechin and quercetin in aesculapiaceae species by HPLC. Pharmacologyonline, 2009; 3:837-846.
15. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute, 1990; 82:1107-1112.
16. Surana SJ, Tatiya AU, Kalaskar MG. Identification of Bioactive Compounds and Possible Mechanism of Hepatoprotective Activity of *Ficus microcarpa* L. Fil. Bark Extracts in Ethanol-Induced Chronic Hepatic Injury in Rats. Indian Journal of Pharmaceutical Education and Research. 2017; 51:706-712.
17. Rodríguez M, Estrela J, Ortega Á. Natural polyphenols and apoptosis induction in cancer therapy. Journal of Carcinogenesis & Mutagenesis S, 2013, 6:1-10.