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Research Article

IN VITRO ANTIOXIDANT STATUS, TOXICOLOGY AND ANTITUMOR ACTIVITY OF *ACACIA CAESIA* EXTRACT AGAINST DALTON'S ASCITIC LYMPHOMA (DAL) INDUCED TUMOUR MODEL

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ABSTRACT

Acacia caesia (L.) Willd., is an armed woody straggling shrub abundantly distributed in the foot hills of the Western Ghats. It is an ethnomedicinal plant used to cure skin, sexual problems, wound, stomach and tooth problems. Different effects of ethanolic extract on Dalton's Ascitic Lymphoma (DAL) induced tumor inoculation as well as its hematological parameters have been examined for the first time. The acute oral toxicity study was done by using Wistar rats according to OECD guidelines 423 for that the animals were divided into 6 groups of 6 animals each. The anti-tumor activity of the ethanolic leaf extract of *Acacia caesia* was determined by injecting DAL cell suspension in to the peritoneal cavity of the animals and treatment was started after 24 hours of the tumor inoculation continued once daily for 14 consecutive days and the efficacy of test sample was compared with that of 5-Fu (Flurouracil) and DAL control. The results of *invitro* antioxidant status revealed that the highest DPPH radical scavenging potential as indicated by the lowest IC_{50} concentration was recorded for the ethanol extract (IC_{50}17.80 µg/ml). Also, the same extract exhibited strong TAA equivalent on $ABTS^{\bullet+}$(4387.5±169.5 µ mol g^{-1}). The acute toxicity study on test animals at different dose levels showed no significant changes in behavior before and after the administration of an oral dose of test plant extract. Further treatment with test extract significantly decreased the development of tumor volume, tumor packed cell volume and percentage increase in body weight when compared to DAL induced tumor control group, also increasing the life span, restoring the total white blood cell count and hemoglobin. The treatment with test extract at doses of 25 and 50 mg/kg normalized the body weight and hematological parameters. The outcome of the present work indicates that *Acacia caesia* extract could be used as natural anticancer agent for human health.

Keywords: *Acacia caesia,* Dalton's Ascitic Lymphoma (DAL), DPPH radical, $ABTS^{\bullet+}$

1. INTRODUCTION

According to the World Health Organization [1, 2] cancer today is one of the major causes of fatality worldwide. The World Health Organization has estimated that deaths from cancer worldwide are projected to continue rising with an estimated 13.1 million deaths in 2030 [3]. Oxidative stress is stimulated by different type of free radicals which has been implicated in the pathology of several sicknesses such as inflammatory conditions, cancer, diabetes and aging [4]. Nowadays, the new innovative techniques like nano-therapy, neutron capture, low-intensity electro resonance therapy have developed for curing oncological illness, together the classical methods like chemotherapy, surgery and radiation therapy are used for further treatment. Though, all of the above ways are accompanied by a number of side effects, which also negatively affect the patient's health [5]. As a result, the development of the cancer drugs from the plant materials and their use in the clinical practice are still today an urgent task [6, 7]. For experimental anticancer drug discovery using tumor models have an extensive part. Among all a Dalton's Ascites Lymphoma model Swiss albino mice indicate a suitable model system to learn about antitumor property [8].

Plants rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, neurodegenerative diseases, inflammation and problems caused by cell and cutaneous aging [9]. Traditional healers of different regions in India particularly Chhattisgarh used *Acacia* species for treatment of various cancer types [10]. The current scientific investigation exhibit that a variety of food plants, medicinal plants and spices containing primary

and secondary metabolites are very much helpful in the treatment of different cancer types such as lung, stomach, skin, breast, cervix, liver and prostate cancers [11]. From the current data, more than 3,000 plants worldwide have anticancer properties. Based on the scientific viewpoint, the idea of the anticancer herbal raw materials searching is not new, but for the correct user, the detailed phytochemical and pharmacological studies are always necessary. Thus, the present research results undoubtedly provide new knowledge about the anticancer effect.

Acacia species is one of the richest resources of bioactive flavonoids, alkaloids, phenolics, saponins, polysaccharides, tannins and terpenoids [12, 13]. The published reports of various biological activities of *Acacia* species include hypoglycemic, anti-inflammatory, antitumor [14], antifungal [15], antiplatelet aggregation, spasmogenic and vasoconstrictor, antihypertensive, anti-hepatitis C virus [16] antioxidant potential [17], wound healing [18], antinociceptive [19], chemopreventive and antimutagenic [20] and finally anthelmintic activity [21]. Several bioactive agents have been identified from the various species of *Acacia* which includes androstene steroid, gallic acid, ellagic acid, quercetin, isoquercitin, kaempferol, naringenin, rutin, lupane, niloticane, umbelliferone and catechin [22]. Among the *Acacia* species, *Acacia caesia* occupies an imperative place in the indigenous system of medicine against various diseases. However, no antitumor activity against DAL induced ascitic tumor has been reported previously for *Acacia caesia* species. Therefore, the present study was planned to explore the possible *in vivo* antitumor activity of leaf extract of *A. caesia* against DAL induced ascitic tumor in Swiss albino mice.

2. MATERIAL AND METHODS

2.1. Chemicals and instruments

The chemicals and instruments used for *in vitro* antioxidant, toxicology and Daltons Ascites Lymphoma (DAL) studies were procured from the Precision Scientific Company, Coimbatore, India. All analytical grade solvents were obtained from E-Merck Ltd, Mumbai, India. The 5-Fu (Flurouracil), which is used for anti-cancer activity was obtained from Sigma chemicals.

2.2. Plant material

Fresh and healthy leaves of *A.caesia* were collected from the Maruthamalai Hill (arid; 540 m above msl; dry deciduous forest), Coimbatore District (a part of the Western Ghats) during the month of June - December, 2017. Also, the plants were collected in their flowering and fruiting seasons from the natural habitat. The collected study plant was identified with the help of the existing Floras [23-25] and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu (Voucher specimen No. BSI/SRC/5/23/2015/TECH/343). The collected plant specimens were pressed properly by using the method [26] and the preserved specimen was deposited for future reference at the Department of Botany, Vellalar College for Women (Autonomous), Erode, Tamil Nadu, India.

2.3. Shade drying, powdering and sample preparation

Freshly collected leaves were cleaned to remove adhering dust and then shade dried. The shade dried leaves were mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and extracted in Soxhlet apparatus successively with different solvents in the increasing order of polarity. Each time, before extracting with the next solvent, the powdered material was dried in a hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 hrs and the water extract was filtered. The obtained extracts were combined, filtered and dried in a rotary evaporation apparatus at 80°C. The powders were stored in airtight containers at 4°C for further use [27].

2.4. *In vitro* antioxidant assay

The free radical scavenging activity on $DPPH^{\bullet}$ [28] and antioxidant activity by radical cation $ABTS^{\bullet+}$ [29] was performed by using BHT and Trolox positive controls and simultaneously calibration curve was plotted to know the amount of antioxidants present in the extract.

2.4.1. Experimental animals

The Wistar rats mass ranging from 150-200 g of male breed were used for toxicity studies and Swiss female albino mice weighing 20±5 g were used for *in vivo* cancer studies and were obtained from Animals Breeding Station, Mannuthy, Thrissur. All the animal experiments were performed according to the guidelines of the Institutional Animal Ethical Committee, Govt. of India (Reg No. 722/02/a/CPCESEA).The animals were fed with standard pellet diet (Sai Durga feeds, Bangalore, India) and water *ad libitum*. They were maintained in

controlled environment (12:12 h light/dark cycle) and temperature (30±2°C).

2.4.2. Behavioural and toxicological effects

The behavioural toxicity study of the extract of *A. caesia* was evaluated in Wistar rats as per OECD guidelines. Before that the test animal Wistar rats received ethanol extract at various doses (1000-5000 mg/kgbw.) orally. They were observed for the toxic symptoms continuously for first 4 h after dosing, and finally the number of survivors was noted down after 24 h. In the toxicity study, no mortality occurred within 24 h under the tested doses of EAC.

2.5. Cell line

The DAL cell line was obtained from Amala Cancer Research Institute (Thrissur, India) and was propagated into transplantable tumors in the peritoneal cavity of Swiss albino mice. The freshly aspirated cells from the mouse peritoneum were washed with Phosphate Buffer Saline (PBS) under sterile conditions and their concentration was determined using a hemocytometer before transplantation. Animals were inoculated with 1×10^6 cells/mouse.

2.5.1. DAL - induced ascitic tumor studies

In order to determine the influence of EAC extract on the hematological status of DAL bearing mice on five groups (n= 6) of mice. All the groups except control were inoculated with DAL (1×10^6 cells/mouse) and Group I, served as the normal control, Group II was treated with singly DAL, Group III, served as DAL along with 5-FU standard drug at the dose of 20 mg/kg (i.p.) b.w, which served as a positive control. Group IV and V were plant extract treated groups with concentration at 25 and 50 mg/kg b.w. respectively. All the treatments were given IP at 24 hours after DAL tumor inoculation and continued for 14 consecutive days.

2.5.2. Determination of Body weight, Tumor volume, Tumor packed cell volume and Mean survival time

Body weight: All the mice were weighed for every five days, after tumor inoculation the average gain in body weight was determined. And also, a percentage of decrease in body weight was calculated. The body weight (BW) of the control and treated groups were measured from week intervals (3 weeks). ***Tumor volume and weight:*** After 14 days of treatment, mice were dissected and the ascetic fluid was collected from peritoneal cavity. The volume was measured by taking it in a centrifuge tube and weighed immediately. ***Tumor packed cell volume:*** The ascetic fluid was collected into Wintrobe's tube and it was centrifuged at the rate 3000 rpm for a period of one hour. The volume of packed cells read directly as percentage. ***Mean survival time:*** The antitumor effect of *A.caesia* was determined by monitoring the death pattern of animals due to tumor burden using mean survival time. After induction of DAL, every day checks all the groups for mortality and record how many days the mice are survived the mean survival time (MST) was calculated by using the following formula: Mean survival time = [1st Death + Last Death]/2.

2.6. Haematological Parameters

After 14 days, the blood was collected from each group of animals by using tail vein method. The hematological parameters like total RBC, WBC counts, total Hemoglobin content (Hb), Packed Cell Volume (PCV), Percentage of polymorphs, lymphocytes, monocytes, eosinophils and Mean Cell Hemoglobin (MCH) were estimated [30-33].

3. RESULTS AND DISCUSSION

3.1. DPPH$^{\bullet}$and ABTS$^{\bullet+}$scavenging activity

Oxidative stress has been implicated in etiology of a number of human ailments [34]. Fig. 1 shows the DPPH$^{\bullet}$ scavenging activity of petroleum ether and ethanol extracts of *A. caesia* at five different concentrations in the reaction mixture. All the extracts exhibited dose dependant increase in activity. The highest DPPH radical scavenging potential as indicated by the lowest IC_{50} concentration was recorded for the ethanol extract (IC_{50}17.80 µg/ml) followed by petroleum ether extract (190.95 µg/ml). This sample compares well with the BHT standard (IC_{50} 2.083 µg/ml) in minimum concentrations. The ABTS radical cation scavenging activity of the petroleum ether and ethanol extracts of *A. caesia* is represented in Table 1. The activity of the tested sample extracts was expressed as Trolox equivalent - the micromolar Trolox solution having an antioxidant capacity equivalent to 1 g dry matter of the substance under investigation. All the samples exhibited ABTS$^{\bullet+}$ scavenging activity, the petroleum ether extract exhibited strong TAA (4387.5±169.5 µ mol g^{-1}) followed by ethanol extract with TAA values (1803.2± 220.9µ mol g^{-1}). Similar oxidative status was noted and recorded [35, 36].

Table 1: ABTS$^{\bullet+}$ scavenging activity of different solvent extracts of *Acacia caesia*

Sample	TAA* (µM Trolox equivalent/g-1 extract)[#]
Petroleum ether	1803.2 ± 220.9
Ethanol	4387.5 ± 169.5

[#]*Values are expressed as means of triplicate determinations ± Standard Deviation*

***** *Total antioxidant activity (µ mol equivalent Trolox performed by using ABTS$^{\bullet+}$)*

Fig. 1: DAL induced Ascitic lymphoma (DAL)-Antitumor Model

3.2. Cell line

3.2.1. Determination of EAC on body weight

Swiss female albino mice treated with plant extract at a dose of 50 mg/kg showed significant ($P<0.001$) reduction in percent increase in body weight of animals, as compared to DAL tumor-bearing mice (Table 2). The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals [37].

3.2.2. Effect of EAC on tumor growth

The effect of EAC on tumor growth response was shown in table 2, after treatment with EAC (25 and 50 mg/kg) reduced significantly the tumor volume and tumor packed cell volume in a dose dependant manner as compared to that of the DAL control group. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meetthe nutritional requirement of tumor cells [38].

3.2.3. Effect of EAC on mean survival time

The effect of EAC on mean survival time was shown in table 2, on oral treatment of EAC to the tumor induced DAL mice, the mean survival time of control group was found to be 24±0.00, where it was increased to 17.83± 1.85 (EAC 50 mg/kg) respectively in EAC treated groups and whereas the standard drug 5-fluorouracil (20 mg/kg) treated group had a mean survival time19.16±1.57. Similarly, Olha Mykhailenko *et al.* suggested that the Iridaceae family plants water dry extracts are promising anticancer reagents.

3.3. Effect of EAC on hematological parameters

The effects of EAC on hematological parameters were shown in table 3. The hematological parameters were

significantly (P<0.001) altered after 14 days of treatment when compared with the DAL control group. Compare to standard and plant extracts treated group the total WBC count had significantly increased (P<0.001) in the DAL control group (24.23±2.10). Whereas, the RBC count, hemoglobin and platelets were decreased in the DAL control cell group. After treating for 14 days with ethanolic extract of test plant at doses of 25 and 50 mg/kg, the body weight and hematological parameters had normalized and close to the normal group. The WBC was significantly (P<0.001) decreased in plant extracts treated groups and RBC was significantly (P<0.01) increased in plant extracts treated groups. Similarly, hemoglobin significantly (P<0.001) increased in plant extracts treated groups. Polymorphs and Monocytes were significantly (P<0.01) increased in DAL control group whereas lymphocytes were decreased in the DAL control cell group (Fig. 2). Eosinophils and MCH (Mean Cell Hemoglobin) counting showed slight differences, when compare to control group. Swapna *et al*. showed that the methanolic extract of *Phyllanthus polyphyllus* (MPP) possess significant antitumor and cytotoxic activity on DAL and human cancer cell lines.

Table 2: Effect of ethanolic extract of *A. caesia* on Body weight, Tumor volume, Tumor packed Cell volume and Mean Survival Time against Daltons Ascites Lymphoma induced cancer in Swiss albino Mice

Group	Body weight		Tumor Volume (ml)	Tumor Packed Cell Volume (%)	Body Weight – Week intervals			Mean Survival Time
	Initial	Final			1st Week	2nd Week	3rd Week	
Control	20.16±0.30	23.50±0.61	0.00±0.00	0.00±0.00	121±0.00	138±0.00	141±0.00	24±0.00
Only DAL	24.33±0.21	25.33±9.74	25.66±0.76***	18.21±0.76***	161±0.00	189±0.00	134±0 .00	16.33±1.30**
DAL +STD 20 mg/kg	24.83±0.47	18.16±8.27	8.333±1.47***	6.33±1.47***	145±0.00	184±0.00	159±0.00	19.16±1.57^{ns}
DAL+EXT 25 mg/kg	22.00±0.51	24.33±10.9	20± 1.46***	17±1.46***	137±0.00	160±0.00	126±0.00	17.33±1.25**
DAL+EXT 50 mg/kg	24.16±0.47	23.33±7.57	9± 0.365***	7±0.36***	131±0.00	211±0.00	91±0.00	17.83±1.85*

*Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's ***P<0.001, **P<0.01, *P<0.05 calculated by comparing treated group with Control group.*

Table 3: Effect of ethanolic extract of *A. caesia* on the Haematological Parameters (Complete Blood Count)

Group	Control	Only DAL	DAL + STD	DAL+EXT 25 mg/kg	DAL+EXT 50 mg/kg
Total RBC Count (x10^6µL)	5.91±0.24	4.96±0.21^{ns}	4.33±0.38**	4.69±0.33*	5.26±0.30^{ns}
Total WBC Count (x10^3µL)	10.43±0.38	24.23±2.10***	17.56±0.91***	19.4±1.10***	11.2±0.67^{ns}
Total Haemoglobin(Hb)	14.76±0.73	10.53±0.60**	0.9±0.25***	11.06±0.99**	12.86±0.91^{ns}
Packed Cell Volume (PCV)	45.3±2.21	32.6±1.80**	28±0.76***	33.86±2.79**	39.66±2.74^{ns}
Polymorphs (%)	0.5±0.73	15.66±1.80***	10.33±0.55**	13.33±0.21***	2.66±0.42^{ns}
Lymphocytes (%)	87.66±1.68	75±2.22	83±1.31	80.66±0.55	90±0.00
Monocytes (%)	3.33±0.42	4.66±0.55	0.2±0.36	0.3±0.63	2.66±0.21
Eosinophils (%)	0.4±0.73	4.66±0.21	4.66±0.55	0.3±0.36	4.66±0.21
MCH(Mean Cell Hemoglobin) (Pg)	25.06±0.51	24.46±0.29^{ns}	23.43±0.17*	24.4±0.44^{ns}	25.2±0.33^{ns}

*Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's***P<0.001, **P<0.01, *P<0.05 calculated by comparing treated group with Control group.*

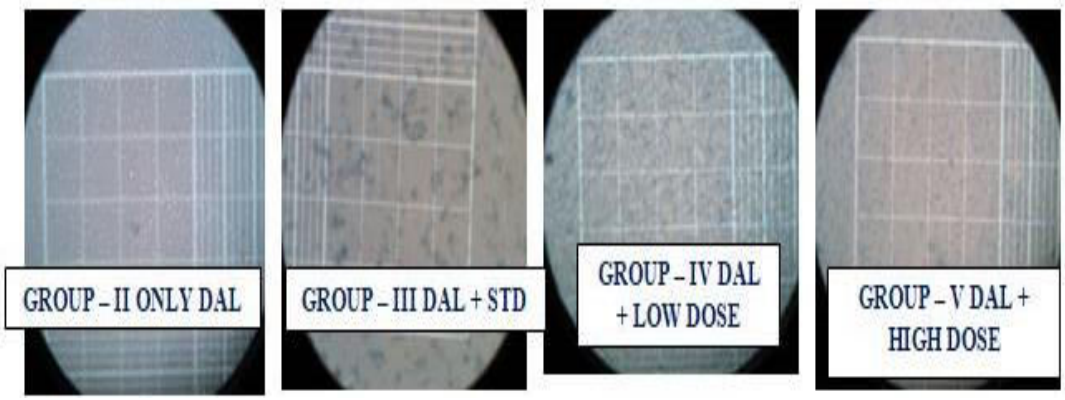

Fig. 2: DAL induced Ascitic lymphoma (DAL)-Antitumor Model (Viable and Non-viable cells)

4. CONCLUSION

The findings of this study support the fact that *A. caesia* possess promising sources of potential antioxidants. The toxicity studies on test animals clearly indicate that no mortality was occurred within 24 h under the tested doses of EAC. There is no doubt that the EAC treated group had significant ($P<0.001$) activity to inhibit tumor growth induced by the DAL cell line. Haematological study of *A. caesia* proved statistically significant ($P<0.001$, $P<0.01$, $P<0.05$) values. The study finally concluded that the study plant *A. caesia* has potential in the development of anticancer therapy and further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-cancer studies.

Conflict of interest

The authors declare that they have no conflicts of interest.

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6. REFERENCES

1. Cancer. WHO, Geneva, Switzerland. Available via https://www.who.int/news-room/fact-sheets/ detail/cancer (Accessed 19 November 2019); 2019.
2. Lang-Schwarz C, Melcher B, et al. *Hum. Pathol.,* 2019; **89**:81-89.
3. Merel K, Stephen J, David K, Helen M. *Asian Pac. J. Cancer Prev.*, 2012; **13**:421-425.
4. Marx JL. *Science,* 1987; 235:529.
5. Edy Meiyanto, Adam Hermawan, Anindyajati. *Asian Pac. J. Cancer Prev.,* 2012; **13**:427-436.
6. Sawadogo WR, Schumacher M, et al. *Biochem. Pharmacol.*, 2012; **84**:1225-1240.
7. Wang X, Chen Y, Fang Z. J. *Photochem. Photobiol. B: Biol*, 2019; **204**:111587-111601.
8. Shanker A, Singh SM. Sodhi A. *Tumor Biol.*, 2000; **21**:288-298.
9. Pratt DE. Antioxidants and cancer prevention I (ACS Symposium Series 507) edited by M. Hang, C. Ho & C Le (American Chemical Society, Washington DC), 1992; 54.
10. Kalaivani T, Mathew L. *Food Chem.Toxicol.*, 2010; **48**:298-305.
11. Asowata-Ayodele AM, Afolayan AJ, Otunola GA. *S. Afr. J. Bot.,* 2016; **104**:69-75.
12. Seigler DS. *Biochem. Syst. Ecol.*, 2003; **31**:845-873.
13. Sharmila S, Ramya EK. *Asian J. Pharm. Clin. Res.*, 2019; **12(1)**:520-528.
14. Lam SK, Ng TB. *Phytomedicine*, 2010; **17**:621-625.
15. Lopes V, Moraes R, Araujo B. *Phytochemistry,* 2009; **70**:871-879.
16. Lee JC, Chen WC, Wu SF. *J. Virol. Antivir Res.*, 2011; **89**:35-42.
17. Singh R, Singh B, Singh S. *Food Chem.*, 2010; **120**:825-830.
18. Tung YT, Wu JH, et al. *Food Chem.,* 2008; **115**:1019-1024.
19. Dongmo AB, Nguelefack T, Lacaille D. *J. Ethnopharmacol.*, 2005; **98**:201-206.
20. Meena PD, Kaushik P, Shukla S. *Asian Pac. J. Cancer Prev.*, 2006; **7**:627-632.

21. Bachaya HA, Iqbal Z, Khan MN, et al. *J. Ethnopharmacol.*, 2009; **123**:325-329.
22. Sharmila S, Ramya EK, et al. *Int. Res. J. Pharm.* 2019; **10(5)**:145-149.
23. Gamble JS, Fischer CEC. Flora of the Presidency of Madras. Botanical Survey of India, Calcutta, 3 Vol. 1957.
24. Fyson PF. The Flora of the Nilgiri and Pulney hill tops. Superintendent, Government Press, Madras, 3 Vol. 1915-20.
25. Matthew KM. The Flora of the Tamil Nadu Carnatic. The Rapinet Herbarium, St. Joseph's College, Tiru-chirapalli, 1983; **3**:278-279.
26. Jain SK and Rao RR. Hand Book of Field and Herbarium Methods. New Delhi.1970.
27. Harborne JB. Phytochemical methods, Chapman and Hall, London (1st ed.), 1973; 33-35.
28. Blios MS. *Nature,* 1958; **26**:1199-1200.
29. Re R, Pellegrini N, et al. *Free Radical Biology and Medicine*, 1999; **26**:933-956.
30. Lowry OH, Rosebrough NJ, et al. *J. Biol. Chem.,* 1951; **193(1)**:265-275.
31. Docie JV. In: Practical Haemotology, London, J&A Churchill Ltd, 1958; 38-42.
32. D'Amour FF, Blood FR, Belden DA, In: The Manual for Laboratory Work is Mammalian Physiology (The University of Chicago Press, Chicago. 1965; 148-150.
33. John MB. Laboratory medicine hematology 4th edition, CV Mosby Co ST Louis, 1972; 1198-1204.
34. Amarlal S, Mira L, et al. *Bioorg. Med. Chem.,* 2009; **17(5)**:1876-1883.
35. Krishnaveni M, Amsavalli L, et al. *Int. J. Pharm. Sci. Rev. Res.*, 2013; **21(1)**:160-163.
36. Kalaichelvi K, Sharmila S, Dhivya SM. *Asian J. Pharmaceu. Clinical Res.,* 2018; **11(1)**:105-110.
37. Clarkson BD and Burchenal JH. *Prog Clin. Can.,* 1965; **1**:625-629.
38. Prasad SB and GiriA. *Ind. J. Expt. Biol.,* 1994; **32**:155-162.
39. Olha Mykhailenko1, Roman Lesyk, et al. *J. Appl. Pharm. Sci.* 2020; **10(7)**:59-63.
40. Swapna G, et al. *Der Pharmacia Sinica.*, 2014; **5(2)**:23-28.