



RESEARCH ARTICLE

# Immunomodulatory and anti-inflammatory effects of active compounds of *Cassia angustifolia* Vahl. leaf extract

Walaa Najm Abood

Department of Microbiology, College of Vet. Medicine, University of Diyala, Baquba 32001, Iraq

\*Email: [walaaabood@gmail.com](mailto:walaaabood@gmail.com)



## ARTICLE HISTORY

Received: 03 December 2023

Accepted: 02 January 2024

Available online

Version 1.0 : 02 April 2024



## Additional information

**Peer review:** Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

**Reprints & permissions information** is available at [https://horizonepublishing.com/journals/index.php/PST/open\\_access\\_policy](https://horizonepublishing.com/journals/index.php/PST/open_access_policy)

**Publisher's Note:** Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Indexing:** Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See [https://horizonepublishing.com/journals/index.php/PST/indexing\\_abstracting](https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting)

**Copyright:** © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

## CITE THIS ARTICLE

Abood WN. Immunomodulatory and anti-inflammatory effects of active compounds of *Cassia angustifolia* Vahl. leaf extract. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.3159>

## Abstract

*Cassia angustifolia* medicinal plant used for the treatment many diseases such as respiratory diseases, skin inflammation, and has the ability of anti-bacterial and anticancer properties. In this work was investigated the Immunomodulatory and anti-inflammatory effect of *C. angustifolia*. Methods: The ethanol leaf extract of *C. angustifolia* was utilized to examine gene expression related to angiogenesis cytokines in treated RAW 264.7 macrophage cell with *C. angustifolia* leaf extract. The results demonstrated increasing significantly in the treated macrophage RAW 264.7 viability accompanied by an improvement in angiogenesis cytokines expression with the inhabited Nitric Oxide production as dose depended manner. GCMS analysis was identified 11 active components within the extract, each exhibiting biological activity as antioxidant, antitumor and anti-inflammatory, Notable compounds include hexadecanoic acid, 2 Pentadecanone, Phthalic acid, oxalic acid, carbonic acid, Tricosane, Undecanal and many others. Conclusion: Ethanol leaf extract of *C. angustifolia* exhibits the immunomodulatory and anti-inflammatory effect through inhibition NO production and increasing angiogenesis cytokines expression.

## Keywords

anti-Inflammatory bioactive compounds; *Cassia angustifolia* ; immunomodulation

## Introduction

Traditional plants were widely use in recent years to treat a lot of diseases instead chemical drugs with fewer side effects that may be effective throughout the use of chemical drugs for a long time. *C. angustifolia* is one of the traditional medicines with the common name senna in Makkah the city in the Saudi Arabia country was discovered and used in the different place in the world for pharmacological purpose (1).

*C. angustifolia* is used in the pharmacological treatment for many diseases as respiratory inflammation, skin infection, piles, and heart disease (2). The research application used *C. angustifolia* extract for antifungal and antibacterial (3, 4). Other study was applied *C. angustifolia* leaf extract for treatment hemorrhoid and colon inflammation (5). In the pharmacological purpose different parts from *C. angustifolia* plant are used in the medicine as leaf, roots, and seeds. That diagnosed have a lot of active compounds like flavonoid, phenol, alkaloids, naphthopyrone glycosides and investigated for their biological activities: anti-inflammation, anti-oxidants, hepatoprotective, and antimicrobial (6-8).

The immune response is the interacting between innate and specific response to immune process. Phytochemical have an effect on the immune responses through enhanced modulation in the one or more components of immune response (9). Immunomodulation compounds like alkaloids, phenols, polysaccharides, and lectins (10). Many plants were investigated for their immunomodulatory effect as *Aloe vera*, *Allium sativum*, *Curcuma longa*, *Centella asiatica*, *Piper longum*, and *Ocimum sanctum* [11]. One of the modulation effect enhanced apoptosis process (12).

In previous study was showed the Immunotoxicity of *Senegalia greggii* seed extract by effect on the CD8 Tcells, CD4 Tcell, the level of TGF-beta and TNF-alpha and effect on the inflammatory process (13). A lot of countries dependent on the herbal medicine in the cure of many diseases like China, Japan, Iraq, Egypt, and India, which play an important role in the modulation of immune system (14). Phytochemical polysaccharide in the medicinal plants act as anti tumor (15). Methanol extract of *Enicostema axillare* effect on both cell mediated and humoral immune process by decreasing pro inflammatory mediators in the macrophages (16). Given the significant role of the important *C. angustifolia* in medicine used in the different field, this study was aimed investigated the immunomodulatory and anti inflammatory influence ethanol leaf extract of *C. angustifolia*.

## Materials and Methods

### Plant extraction

*C. angustifolia* also common known as (*Senna alexandrina* Mill) leaf Figure 1, was purchased under voucher number (EM3.12). The ethanol extract was done as described by Abood *et al.* (17).

### Immunomodulation effect of *C. angustifolia*



**Fig. 1.** *Cassia angustifolia* (*Senna alexandrina* Mill).

The immunomodulatory effect of ethanol leaf extract of *C. angustifolia* was investigated on the effect RAW 264.7 macrophages cells via measuring cell viability after treatment with different doses from extract at different time exposure. The final concentration of extract was 125, 250, 500 and 1000  $\mu\text{g}/\text{mL}$ , the method was done as described by Abood (9).

### Effect of *C. angustifolia* on the production of Nitric oxide

The impact of *C. angustifolia* on the production of Nitric oxide (NO) from macrophage RAW 264.7 cell was investigated by exposed the cell to graded concentration doses 25, 50, 100, 200  $\mu\text{g}/\text{mL}$  from *C. angustifolia* ethanol leaf extract by applied Griess method (18). Stimulation of RAW 264.7 cell at the number  $1 \times 10^6$  cells / well by incubated with LPS (100  $\mu\text{g}/\text{mL}$ ) with extract in the plate at 24h at 37°C with 5%  $\text{CO}_2$ . The cells were treated with LPS be as positive control. A dilution of  $\text{NaNO}_2$  was prepared as standard curve. Aspirated culture media and centrifuged at 4000 r.p.m for 10 min. Griess reagent was added to the an equal amount from supernatant and incubated at room temperature in the dark place at 30 min, at the end of the time read the absorbance was at 546 nm.

### Anti-inflammatory effect of *C. angustifolia*

To investigated anti inflammatory influence leaf extract of *C. angustifolia* through initiated angiogenesis process to produce many angiogenesis expression proteins and cytokines in the excited macrophage RAW 264.7 cell line (from Raybio C-series angiogenesis, USA).

Method involves treating the cells in the 100  $\mu\text{g}/\text{mL}$  *C. angustifolia* leaf extract, 24h. Membrane for Antibody array placed in the tray then two mL block buffer added buffer for thirty min, the end of the incubation time removed it by aspiration. One mL from media to treated cell is added to antibody array membrane, incubated to three hours at room temperature in the end of incubated time was aspirated. Membrane was washed in the 1X wash buffer 1 by 2 mL for 5 min at room temperature, replicated wash buffer three times. Repeated wash step by wash buffer 2 as the same step for wash buffer 1. Added 1 mL from biotinylated antibody on the membrane well, incubated 2 h at room temperature and removed at the end of incubation. Repeated the wash step. HRP-streptavidin 2 mL in the 1X concentration is inserting on membrane then incubated to 2h at room temperature and removed at end of incubation, repeat wash step. Then transfer membrane to the plastic sheet, 500  $\mu\text{L}$  from detection buffer added and a chemilluminescence imaging system was employed to obtain the results.

### Diagnosis of active compounds

Leaf *C. angustifolia* extract was applied for gas chromatography mass spectrum analysis, is done by MSDCHEM device. Phytochemical analysis is done through inoculation 0.5  $\mu\text{L}$  extract, and divided ratio was 1:50, at 250 temperature for injection and back eluent. Flow column 1mL/min. Helium used as carrier gas and the temperature oven at 60 to 246. Collected rate is 4 C/ min.

Detection active compounds through spectrum for the compound and compared with willy and NIST/EPA/NIH mass spectral library.

### Analysis of result

Data were presented as (mean  $\pm$ SD), statistical analysis of result was done using T-test and significant with P value < 0.05. Analysis for gene expression of angiogenesis and pro-

tein array was done through C-Series angiogenesis analysis manufacture software.

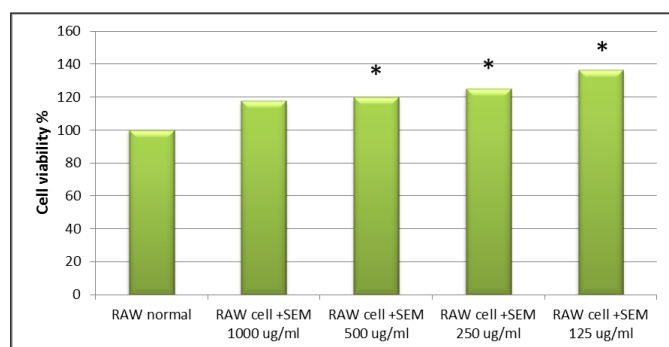
## Results

### Immunomodulatory effect for *C. angustifolia* leaf extract

Immunomodulatory effect for ethanol *C. angustifolia* leaf extract on the treated RAW 264.7 cell line was showed increased in the viability of the cells significantly at P value < 0.05 to dose dependent manner compare to normal not treated cells as presented in the Figure 2.

### Effect of *C. angustifolia* on the production of Nitric oxide (NO)

The impact of *C. angustifolia* on the production of Nitric oxide from treated macrophage RAW 264.7 cell showed the production of Nitric oxide was decreased significant at P

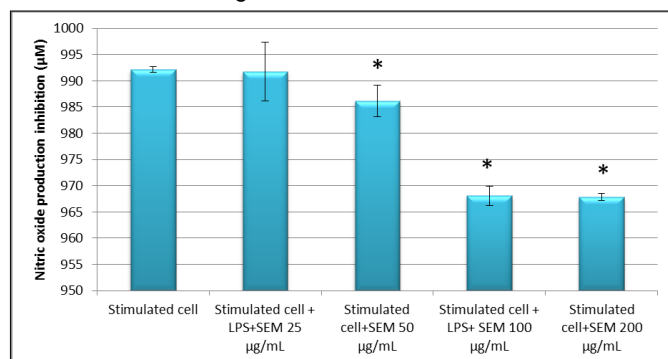


**Fig. 2.** The immunomodulatory effect of *Cassia angustifolia* leaf extract for treating RAW 264.7 cell. \*Significant at  $P < 0.05$ .

value < 0.05 in the stimulated RAW 264.7 cells for different doses from leaf extract at dependent manner compared with non-stimulated RAW 264.7 cell, presented in the Figure 3.

### Anti-inflammatory effect of *C. angustifolia*

The application of *C. angustifolia* leaf extract to stimulate angiogenesis process to produce many angiogenesis expression proteins on the stimulated macrophage RAW 264.7 cell with *C. angustifolia* leaf extract showed different



**Fig. 3:** The effect of *Cassia angustifolia* on the production of Nitric oxide from treated macrophage RAW 264.7 cell showed the production of Nitric oxide (µM). \*Significant at  $P < 0.05$ .

excite to the angiogenesis expression proteins, increased in the fold of expression for the angiopoietin-1, angiopoietin-2, angiostatin, GM-CSF, I-309, IL-10, IL-1 alpha, IL-1 beta, MMP-1, MMP-9, PECAM-1, Tie-2, TNF-alpha, µPAR, and VEGFR2. While down expression in the angiogenesis expression proteins for Endostatin, GCSF, MCP-4, IL-2, IL-4, I-

TAC, MCP-3, and VEGFR3 (Table 1).

**Table 1:** Angiogenesis expression proteins on the stimulated macrophage RAW 264.7 cell with ethanol leaf extract of *Cassia angustifolia*

Protein	Symbols gene	Fold expression	Fold expression in treated cell with <i>C. angustifolia</i>	Percentage Fold changes
Angiopoietin-1	ANGPT1	2762.675	6770.344	59.19447
Angiopoietin-2	ANGPT2	4834.975	7834.035	38.28244
Angiostatin	CCL1	559277.1	600187.1	6.816214
Endostatin	COL18A1	668347.1	673143.6	0.712558
GCSF	CSF3	33569.43	29067.93	-15.4861
GM-CSF	CSF2	31413.93	33800.4	7.060493
I-309	(TCA-3/CCL1)	99337.08	124980.3	20.51779
IL-10	IL10	108170.6	145961.7	25.8911
IL-1 alpha	IL1A	8907.825	14250.52	37.49123
IL-1 beta	IL1B	10479.43	12006.72	12.72033
IL-2	IL2	2.775	0.05821	-4667.22
IL-4	IL4	2.775	0.05821	-4667.22
I-TAC	(CXCL11)	2.775	0.05821	-4667.22
MCP-3	(MARC/CCL7)	2.775	0.05821	-4667.22
MCP-4	(CCL13)	44840.08	40529.03	-10.6369
MMP-1	MMP1	48422.58	50173.64	3.490018
MMP-9	MMP9	42384.58	49700.98	14.72084
PECAM-1	(CD31)	42624.58	66146.85	35.56069
Tie-2	TEK	24275.58	33289.26	27.07685
TNF alpha	TNF	29879.58	35775.79	16.48103
µPAR	PLG	278274.9	333709.8	16.61171
VEGFR 2	K D R	319074.9	351153	9.135184
VEGFR 3	F L T 4	29899.58	28418.83	-5.21044

### Phytochemical analysis of *Cassia angustifolia*

Phytochemical analysis of *Cassia angustifolia* was done by applied to the GC-MS analysis and the analysis was diagnosis eleven active compounds listed in the Table 2.

## Discussion

Previous studies have extensively documented the diverse biological activities of *C. angustifolia*. This current study

**Table 2:** Phytochemical analysis (GM-CS) of *Cassia angustifolia* leaf extract

Active compound	Formula	RT	Area%
Oxalic acid	C <sub>16</sub> H <sub>30</sub> O <sub>4</sub>	17.15	3.10
2-Pentadecanone	C <sub>18</sub> H <sub>36</sub> O	19.77	4.59
Carbonic acid	C <sub>22</sub> H <sub>44</sub> O <sub>33</sub>	19.77	4.59
Methoxyacetic acid	C <sub>18</sub> H <sub>36</sub> O <sub>3</sub>	20.11	1.98
Tricosane	C <sub>23</sub> H <sub>48</sub>	20.11	1.98
Hexadecanoic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	20.88	7.04
Heptadecanoic acid	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	23.89	2.44
Hexadecyl propyl ether	C <sub>19</sub> H <sub>40</sub> O	24.51	1.41
Undecanal	C <sub>11</sub> H <sub>22</sub> O	26.45	2.47
Octadecanoic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	26.45	2.47
Phthalic acid	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	29.66	31.48



contributes evidence supporting of *C. angustifolia* leaf extract as modulation for immune response by increasing the RAW cells viability through effect on the Nitric oxide production and effect on the expression of angiogenesis protein expression. The angiogenesis process is important in the inflammation through effect in the different roles. Angiopoietin was act synergetic with VEGF to stimulate angiogenesis and enhanced remodeling and controlled of the formation blood vessel (19). Some medicinal plants were investigated for NO modulation like *Linum persicum*, *Dionysia termeana*, *Salvia mirzayanii* and *Ocimum gratissimum* and proven effect to decreased NO production with significant inhibited TNF-alpha and IL-1 acting as anti-inflammatory agents (20, 21). Angiostatin is plasminogen has potential inhibitor endothelial cell proliferation and metastasis of tumor (22). The outcome provide an evidence of the anti inflammatory potential of *C. angustifolia*. The Endostatin has anti cancer effect through tumor regression caused inhibition angiogenesis. The role of GCSF was important in the existence, differentiation and proliferation of neutrophil from heamopoietic, down expression of GCSF was effect on the maturation of neutrophil and synergetic effect on the inflammation process (23, 24). *C. angustifolia* leave extract was leading to increase the production of GM-CSF in the treated cells. The GM-CSF is effect on the development inflammation process, immunomodulator (25).

The effect of *C. angustifolia* leave extract on the modulation production of inflammatory cytokines as IL-10, IL-1 alpha and beta, and TNF-alpha, the modulation caused increasing or decreasing influence for promote inflammation and limiting immune response to pathogens and stimulating macrophage, give T cell development and differentiation (26-28). *Allium cepa* (onion) was consumed through the world, it has many phytochemical as saponins, quercetin, flavonoids, cepanens, phenolic compounds, and organ sulfurs all the antioxidant and immunomodulatory effect by reduced Th cell cytokines but increasing CD4 cells cytokines (29). Chemokines I-TAC is a mediator to the T cells migration and activation and it is role influence by INF-gamma level (30). *Ocimum basilicem* used for treatment respiratory tract infection have anti inflammatory and immunomodulatory potential by increasing INF-gamma and decreasing IL4 in the treated rats (31). In the many of previous studies were showed the pharmacological potential of medicinal plants as anti inflammatory and immunomodulatory agents may be used as drug for treatment many diseases (32).

Additional evidence for immunomodulation of *C. angustifolia* is increasing in the fold expression of MMP family, PECAM-1, TNF alpha, VEGFR2 and  $\mu$ PAR. These inflammations proteins have lot of important and crucial role in the immune response and defense, through play role for recruitment of leukocyte, cytokine production, inflammation process, adhesion of platelet (33, 34). The macrophage plasminogen activators is  $\mu$ PAR (urokinases) that control for phagocytosis to the chronic inflammation leads to immunomodulation and development of immune response by supportive matrix generation and control to

the cells migration, adhesion, proliferation the process in the inflammatory response (35).

## Conclusion

*C. angustifolia* has an important and potential role in the immune response through play the role in the effect on the up and down expression and fold change for angio-genesis proteins. And may be depend in the future drug developed and discover a new immunomodulator therapy as anti-tumor, immunoregulator and anti-diabetic.

## Compliance with ethical standards

**Conflict of interest:** The author declared no conflict of interest.

**Ethical issues:** None.

## References

1. Khan MA, Ahmad M, Zafar M, Sultana S, Marwat SK, Shaheen S, Leghari MK, Jan G, Ahmad F, Nazir A. Medico-botanical and chemical standardization of pharmaceutically important plant of *Tricholepis chaetolepis* (Boiss) Rech. f. Journal of Medicinal Plants Research. 2011;5(8):1471-77.
2. Sultana S, Ahmad M, Zafar M, Khan MA, Arshad M. Authentication of herbal drug Senna (*Cassia angustifolia* Vahl.): A village pharmacy for Indo-Pak subcontinent. African Journal of Pharmacy and Pharmacology. 2012;6(30):2299-308. <https://doi.org/10.5897/AJPP12.446>
3. Akrayi HF. Antibacterial effect of aqueous extracts of spices and herbs against bacteria isolated from frozen meat. Medical Journal of Islamic World Academy of Sciences. 2014;22(1):30-35. <https://doi.org/10.12816/0008168>
4. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicrobial activity. Journal of Pharmacognosy and Phytotherapy. 2016 Feb 29;8(2):25-48. <https://doi.org/10.5897/JPP2015.0367>
5. Naqishbandi A. Plants used in Iraqi traditional medicine in Erbil-Kurdistan region. Zanco Journal of Medical Sciences. 2014;18(3):811-15. <https://doi.org/10.15218/zjms.2014.0038>
6. Singh S, Singh SK, Yadav A. A review on *Cassia* species: Pharmacological, traditional and medicinal aspects in various countries. American Journal of Phytomedicine and Clinical Therapeutics. 2013 Jun 30;1(3):291-312.
7. Hussein H, Al-Khafaji NM, Al-Mamoori AH, Al-Marzoqi AH. Evaluation of *in vitro* antibacterial properties of the crude phenolic, alkaloid and terpenoid extracts of *Cassia senna* L. against human gram-negative pathogenic bacteria. Plant Archives. 2018;18(1):354-56.
8. Agarwal S, Pandey V. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biologia Plantarum. 2004;48(4):555-60. <https://doi.org/10.1023/B:BIOP.0000047152.07878.e7>
9. Porwal O, Ozdemir M, Kala D, Anwer ET. A review on medicinal plants as potential sources of natural immunomodulatory action. Journal of Drug Delivery and Therapeutics. 2021;11(6):324-31. <https://doi.org/10.22270/jddt.v11i6.5125>
10. Parbat AY, Malode GP, Shaikh AR, Panchale WA, Manwar JV, Bakal RL. Ethnopharmacological review of traditional medicinal plants as immunomodulator. World Journal of Biology Pharmacy and Health Sciences. 2021;6(2):43-55. <https://doi.org/10.30574/wjbphs.2021.6.2.0048>

