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Research Article
Effects of Plant Extracts on the Transcriptional Activity of Nuclear Factor-κB

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Abstract

Background and Objective: The Nuclear Factor-κB (NF-κB) family of transcription factors is known to have a central regulatory effect on many physiological processes including the immune response and cell survival. Besides, the modulation of the NF-κB transduction pathway is a principal therapeutic target in cancer and inflammatory disease states. Many natural products have been shown to modulate the transcriptional activity of NF-κB. This research work was aimed at determining the phytochemical content of selected Maltese medicinal plants and investigating their ability to modulate the activity of NF-κB. **Materials and Methods:** Extracts with different polarities were prepared from *Aloe vera* L. (Aloaceae), *Jasonia bocconei* (Brullo) M. Pardo and R. Morales (Asteraceae), *Calendula suffruticosa* Vahl (Asteraceae) and *Ruta bracteosa* DC. (Rutaceae). The extracts were studied for the total content of phenols and alkaloids and DPPH free radical-scavenging activity. Further studies were carried out to investigate extract-induced modulation of the transcriptional activity of NF-κB in human embryonic kidney (HEK293) cells that had been stably transfected with an NF-κB-luciferase reporter construct. **Results:** Extracts from *Ruta bracteosa* caused increased luciferase activity. Besides, significant positive statistical correlations were found between alkaloidal content, increased luciferase activity and increased EC_{50} values for DPPH free radical scavenging activity. **Conclusion:** Polar extracts from *Ruta bracteosa* with high alkaloidal content exerted increased transcriptional activity of NF-κB. Because of the evidence that links apoptotic cell death with activation of NF-κB in a variety of tumor cells, further studies are warranted, so that the chemotherapeutic potential of the plant could be established.

Key words: *Aloe vera*, *Jasonia bocconei*, *Calendula suffruticosa*, *Ruta bracteosa*, HEK293, luciferase activity, DPPH

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

NF-κB is a family of transcription factors of considerable importance that is known to exert its control over the expression of several genes[1]. It is made up of five structurally related proteins p65, p50, p52, c-REL and REL B subunits that are found within the cytosol as inactive heterodimers or homodimers bound to the inhibitory protein, IκB. Activators of NF-κB induce the dissociation of IκB and cause the translocation of the remaining dimer to the nucleus, where it controls the expression of genes that are linked to immunity, inflammation and cell survival. The activation of NF-κB occurs via a variety of stimuli, including inflammatory cytokines, Reactive Oxygen Species (ROS) and bacterial lipopolysaccharide.

Although the activity of NF-κB is essential in normal physiology, various chronic inflammatory diseases and cancers have been associated with the upregulation of NF-κB[2]. An association has been established between inflammation and tumor promotion and progression and NF-κB has emerged as a pivotal component in this link. This is augmented by the upregulation of genes involved in cell proliferation and protection from death and apoptosis which has been associated with aberrant activation of NF-κB during tumor progression[3].

Increasing evidence, however, points to the important role that the activation of NF-κB plays in inhibiting or promoting cell death, depending on cell type and apoptotic stimulus[4-8]. Indeed, it has been shown that NF-κB activation participates in cell death induced by treatment with doxorubicin, paclitaxel, vincristine and vinblastine in a variety of tumor cells including human neuroblastoma and drug-refractory ovarian and breast cancer cells.

Because of the central role which is played by NF-κB in diverse pathological states, the modulation of the NF-κB transduction pathway is a principal target in the treatment of inflammation and cancer. Natural product research has led to the identification of plant-derived phenolic compounds that exert an inhibitory effect on the transcriptional activity of NF-κB[9,10]. Besides, various alkaloids from natural sources have demonstrated either stimulatory or inhibitory activity on this transcription factor[6,7,11].

When screening for biologically active plant constituents, the selection of the plant species to be studied is a crucial factor for the ultimate success of the investigation. The plants that were selected in this study were the following: *Jasonia bocconei* (Brullo) M. Pardo and R. Morales (Asteraceae), *Ruta bracteosa* DC. (Rutaceae), *Calendula suffruticosa* Vahl (Asteraceae) and *Aloe vera* L. (Aloaceae). These plants are indigenous to the Maltese Islands, although doubt exists as to whether *A. vera* is truly indigenous[12]. Based on the role which NF-κB has in the pathology of inflammatory conditions and cancer, these plants were selected on the basis that they belonged to genera that have been shown to exhibit anti-inflammatory properties and cytotoxic activity in tumor cells[13-20]. No studies had previously investigated the effect of extracts from *J. bocconei*, *R. bracteosa* and *C. suffruticosa* on the transcriptional activity of NF-κB. The literature available on the ability of *A. vera* to modulate the activity of this transcription factor is sparse and has been mainly limited to the gel and individual components in the gel and exudate[21-24]. No documentation, however, could be found on the bioactivity of the exudate from the plant in its crude form.

This study aimed to determine the effects of plant extracts on the transcriptional activity of Nuclear factor-κB. In this study, a two-phase approach was designed to reach the aim of the study. The first phase relates to a preliminary study to determine the total phenolic and alkaloidal content. Besides, the ability of plant extracts to scavenge free radicals was used as a measure of the ability of the extracts to attenuate oxidative stress. The results of these studies served as the basis for the second phase of the study in which further *in vitro* investigations were conducted on a human cell line to determine the modulatory effect of the plants on the transcriptional activity of NF-κB.

MATERIALS AND METHODS

Study area: The extractions, phytochemical and pharmacological testing of the extracts was carried out between February, 2010 and March, 2013 at the Department of Clinical Pharmacology and Therapeutics, Faculty of Medicine and Surgery, University of Malta, Malta.

Chemicals and reagents: The following chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA): Petroleum ether (Cat. No. 77399), chloroform (Cat. No. 32211), acetone (Cat. No. 32201), dimethyl sulphoxide (DMSO; Cat. No. 51779), ethanol (96%) (Cat. No. 24106), 2,2-Diphenyl-1-picrylhydrazyl (DPPH; Cat. No. D9132), ascorbic acid (Cat. No. A7506), α-tocopherol (Cat. No. T3251), butylated hydroxytoluene (BHT; Cat. No. B1378), vanillin (Cat. No. V1104). Folin and Ciocalteu's phenol reagent (FC reagent Cat. No. F9252), anhydrous sodium carbonate (Cat. No. 223484), bismuth nitrate pentahydrate (Cat. No. 467839), acetic acid (Cat. No. 695092), potassium iodide (Cat. No. 60400), hydrochloric acid (Cat. No. 84436), disodium sulfide (Cat. No. 407410), nitric acid (Cat. No. 695033), thiourea (Cat. No. 88810), tissue necrosis factor α

Table 1: Maltese medicinal plants and traditional use

Family	Plant	Voucher sample
Asteraceae	*Jasonia bocconei* (Brullo) M. Pardo and R. Morales	RSFS-099
	Calendula suffruticosa Vahl	RSFS-071
Aloaceae	*Aloe vera* L.	RSFS-015
Rutaceae	*Ruta bracteosa* L.	RSFS-362

(TNFα; Cat. No.T0157), dexamethasone (Cat. No. D-1756), DMEM (Cat. No. D5648), hygromycin B (Cat. No. H3274), phosphate-buffered saline (PBS; Cat. No. P3813) and biological grade sodium bicarbonate (Cat. No. S5761). Besides, Fetal Bovine Serum (FBS; Cat. No. 10270), penicillin-streptomycin (Cat. No. 15140) and Trypsin-EDTA (Cat. No. 15400-054) were obtained from Gibco Invitrogen (Thermo Fisher Scientific Inc. Waltham, MA, USA). ONE-Glo® Luciferase Assay System (Cat. No. E6120) and non-radioactive cell proliferation assay (Cat. No. G4000) were obtained from Promega (Madison, WI, USA). The non-radioactive cell proliferation assay kit consisted of a premixed optimized Dye Solution containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Solubilization/Stop solution.

Plant materials and extractions: The aerial parts of the plants were collected on the Maltese Islands between February and October, 2010 and were identified by the authors. Voucher specimens of all the species have been deposited within the Institute of Earth Systems, the University of Malta as illustrated in Table 1. The entire aerial parts of *J. bocconei* and *C. suffruticosa*, including their flower-heads were collected. To conform with ethno-pharmacological data, young shoots of *R. bracteosa* were collected during the pre-flowering phase. In the case of *A. vera*, the leaves were put in the dark at an ambient temperature of 20°C and were left to drain for a total of 6 hrs to collect the exudate. Subsequently, the pulp was manually separated from the rind, homogenized and filtered. The exudate and gel were lyophilized and stored in airtight containers at -40°C until further analysis.

The plant material from *R. bracteosa*, *J. bocconei*, *A. vera* rind and *C. suffruticosa* was dried at a temperature of 39°C in a dehydrator (Kaltenbach; Rommelsbacher ElektroHausgerate GmbH, Germany) and separately extracted twice with petroleum ether, chloroform, acetone, ethanol (96%) and deionized water after maceration for 48 hrs at room temperature. The organic solvents were dried in a ventilated oven at a temperature of 39°C and finally under a stream of nitrogen gas (N-Evap 112; Organomation Associates, Inc., USA). The aqueous samples were lyophilized. The dried extracts were stored in airtight containers at -40°C until analyzed.

Total phenolic content: Total phenolics were determined by a microplate spectrophotometric assay that was developed by Attard[25]. Briefly, the extracts were solubilized in ethanol and transferred to microtiter wells in 5 µL aliquots to which an equal volume of de-ionized water was added. Subsequently, 100 µL of an aqueous dilution (1:10) of FC reagent was added followed by 80 µL of 1 M (anhydrous) sodium carbonate solution. The plate was incubated at 25°C for 20 min in a plate incubator. The intensity of the blue color was read on a microtiter plate reader (Multimode Reader LB940 Mithras; Berthold Technologies, Germany) at a wavelength of 620 nm. Each test was performed in triplicate. Following correction of background absorption by the solvent and extracts, the resultant absorbance (A) was then used to calculate the total phenolic content in the microtiter wells from the regression equation obtained for the construction of the standard calibration curve prepared using 480, 240, 120, 60 and 0 µg mL^{-1} solutions of tannic acid in water. The total content of phenolic compounds was expressed as mg tannic acid equivalents per gram Dry Weight (DW) of extract.

Total alkaloidal content: The spectrophotometric method, which was developed by Sreevidya and Mehrotra[26], was used to estimate total alkaloids. Briefly, to an acidified ethanolic solution of the extract, Dragendorff Reagent was added. The reagent was prepared by the addition of 50 mL of bismuth nitrate pentahydrate 1.6% w v^{-1} aqueous solution containing 20% v v^{-1} acetic acid to 20 mL potassium iodide 40% w v^{-1} aqueous solution. Disodium sulfide was added to the alkaloid complex causing it to release the bismuth which subsequently formed a soluble yellow complex with thiourea in the presence of nitric acid. The amount of bismuth was calculated from a calibration curve constructed for the reaction between different concentrations of aqueous bismuth nitrate pentahydrate solution and thiourea in nitric acid. Since on addition of the Dragendorff reagent, the complex that forms between bismuth and alkaloids follow a stoichiometry of 1:1, the number of moles of bismuth corresponds to that of the alkaloids present. The content of total alkaloids as papaverine equivalents was expressed in mg per gram of dried extract.

Free radical scavenging activity: The capacity of the extracts to scavenge DPPH free radicals was measured by a microplate spectrophotometric assay as described by Lee *et al.*[27] with correction for background interference by the extracts. The standards, ascorbic acid, α-tocopherol and BHT were used as positive controls. Vanillin standard was used to represent poor DPPH free radical scavenging activity[28]. Background interference from carrier solvents and samples was eliminated and the free radical scavenging activity was calculated as follows:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{C-C}_{\text{solvent blank}}\text{-S-S}_0}{\text{C-C}_{\text{solvent blank}}} \times 100$$

where, C represents the absorbance value of DPPH and sample vehicle (DMSO) and S represents the absorbance of DPPH in the presence of the sample. $C_{solvent\ blank}$ represents the absorbance of both sample and reagent vehicles while S_0 represents the absorbance of the sample, sample vehicle and reagent vehicle in the absence of DPPH. The antioxidant activity of plant extracts was expressed as EC_{50}.

Cell line and culture conditions: GloResponse™ NF-κB-RE-luc2P HEK293 cell line (Promega Cat. No. E8520) was used to investigate the effect of the plant extracts on the transcriptional activity of NF-κB. These cells contained a luciferase gene (luc 2P) which is under the control of multiple NF-κB responsive elements. The cells were cultured in a 5% CO_2 humidified atmosphere at 37°C in DMEM supplemented with biological grade sodium bicarbonate, 10% FBS, 1% penicillin-streptomycin and 50 μg mL^{-1} hygromycin B.

Measurement of transcriptional activity of NF-κB: On the day preceding the assay, the medium in the flasks was replenished with growth medium (no hygromycin B). The extracts were solubilized in DMEM 20% ethanol except for the aqueous extract of *J. bocconei* which exhibited preferential solubilization in 20% DMSO. The concentration of the carrier solvent and the highest usable concentrations of extracts in the assay were established in preliminary cytotoxicity assays (see below). GloResponse™ NF-κB-RE-luc2P HEK293 cells were dissociated with Trypsin-EDTA 0.25% v v^{-1} in PBS, suspended in assay medium (DMEM supplemented with 10% FBS) and dispensed at 4×10^4 cells 75 μL^{-1} per well in 96-well white bottom microtiter plates. This was followed by the addition of 5 μL of extract at different concentrations in DMEM 20% ethanol. Dexamethasone 1×10^{-7} M was used as a positive control. The cells were incubated for 30 min at 37°C and treated to a final volume of 100 μL per well with 0.1 ng mL^{-1} TNFα as established in preliminary optimization studies. This was followed by a further incubation period of 5 h in a carbon dioxide humidified atmosphere at 37°C and the addition of 100 μL of One-Glo® Luciferase Assay System Reagent to each well. The plate was incubated at 25°C for 20 min and luminescence was read using a luminometer (LB940 Multimode Reader Mithras; Berthold Technologies, Germany) for 0.5 sec per well. For each concentration of extract, a cell-free control was set up to correct for background luminescence caused by solvent, extract, medium and reagent. Each sample and cell-free control was assayed in triplicate. The luminescence obtained at every concentration of the extract was normalized to the luminescence obtained from cells treated with DMEM 1% ethanol/DMSO only which represented 100% luciferase activity.

Cytotoxicity assay: The cells were cultured as described above and were dispensed at 4×10^4 cells 75 μL^{-1} per well in 96-well microtiter plates. This was followed by the addition of 5 μL of different concentrations of DMSO and ethanol (0-100% v v^{-1}) in DMEM. The cells were incubated for 30 min at 37°C and treated to a final volume of 100 μL per well with 0.1 ng mL^{-1} TNFα as established in optimization studies. This was followed by a further incubation period of 5 hrs in a humidified 5% CO_2 atmosphere at 37°C and the addition of 15 μL Dye Solution containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After a 4 hrs incubation period at 37°C, 100 μL of Solubilization/Stop Solution was added to the culture wells to solubilize the formazan product and the absorbance was recorded at 570 nm using a microplate reader after overnight incubation in the dark at 25°C. A cell-free control was set up for each concentration of the solvent. The absorbance values obtained for the cell-free controls were subtracted from the cell-containing counterparts to correct for background absorption caused by the medium, solvents and reagents. The corrected absorbance values for the increasing concentrations of solvent were normalized to 0% solvent which represented 100% viability. The concentrations of the extracts in the wells before the addition of MTT dye were as follows: 100 μg mL^{-1} for the aqueous and ethanolic extracts of *J. bocconei*; 75 μg mL^{-1} for the ethanolic extract of *R. bracteosa*; 50 μg mL^{-1} for the acetone extract of *R. bracteosa* and *A. vera* exudate and 40 μg mL^{-1} for the acetone extract of *J. bocconei*. At these concentrations, the viability of the cells was higher than 90%.

Statistical analysis: All measurements were conducted in triplicate and average results were reported. Microsoft Office Excel 2007 and XLSTAT (Microsoft, version 19.4.46756, SAS Institute Inc., Marlow, Buckinghamshire, UK) was used for data analysis. The results were analyzed by one-way ANOVA with the Bonferroni post hoc test to compare the statistical difference between means of the data sets and their mean difference. Additionally, for the DPPH assay, EC_{50} values were estimated by a Probit regression analysis using MATLAB (version 7) as suggested by Locatelli *et al.*[29]. Significant differences were considered at $p<0.05$.

RESULTS

The values obtained for the yields of the dry extracts from dried plant material are summarized in Table 2. The aqueous extraction of the four plants yielded the highest extract yield (12.700-28.241% w w^{-1}). The order of decreasing extract yield was aqueous, ethanol, acetone, chloroform and petroleum ether. The lowest yield was obtained for the petroleum ether rind extract with a value of 0.846% w/w. A wide range of values was observed for the phenolic content per gram DW of plant extract (Fig. 1). This ranged from 0.80 mg TAE g^{-1} DW in an aloe gel to 94.87 mg TAE g^{-1} DW in an aloe exudate that was significantly higher ($p<0.01$) than that found in the other extracts. Besides, the phenolic content of the ethanolic and acetone extracts of *J. bocconei* was significantly higher when compared to that found in the remaining extracts ($p<0.05$). Regarding the total alkaloidal content the highest content per gram DW of extract ($p<0.01$) was obtained in the acetone extract of *R. bracteosa* (115.42 mg PVE g^{-1} DW; Fig. 2), which was sequentially followed by the ethanolic and chloroform extracts.

Regarding the antioxidant properties, the ethanolic and aqueous extracts of *J. bocconei* possessed potent antioxidant activities (Fig. 3). No statistically significant differences were found between the EC_{50} values obtained for these extracts and those obtained for α-tocopherol and BHT. Besides, for the ethanolic extract of *J. bocconei*, the EC_{50} was not statistically significantly different from that obtained for ascorbic acid. Other extracts that had low EC_{50} values were the acetone extract of *J. bocconei* and aloe exudate.

Table 2: Yields of the dry extracts from dried plant material

	Yield of dry extract from dried plant material (% ww^{-1})			
	C. suffruticosa	*J. bocconei*	*R. bracteosa*	*A. vera* rind
Aqueous	24.600	12.700	16.690	28.241
Ethanol	10.301	8.175	10.04	3.580
Acetone	2.889	5.969	5.037	0.972
Chloroform	3.760	6.677	6.360	1.539
Petroleum ether	1.269	5.434	2.379	0.846

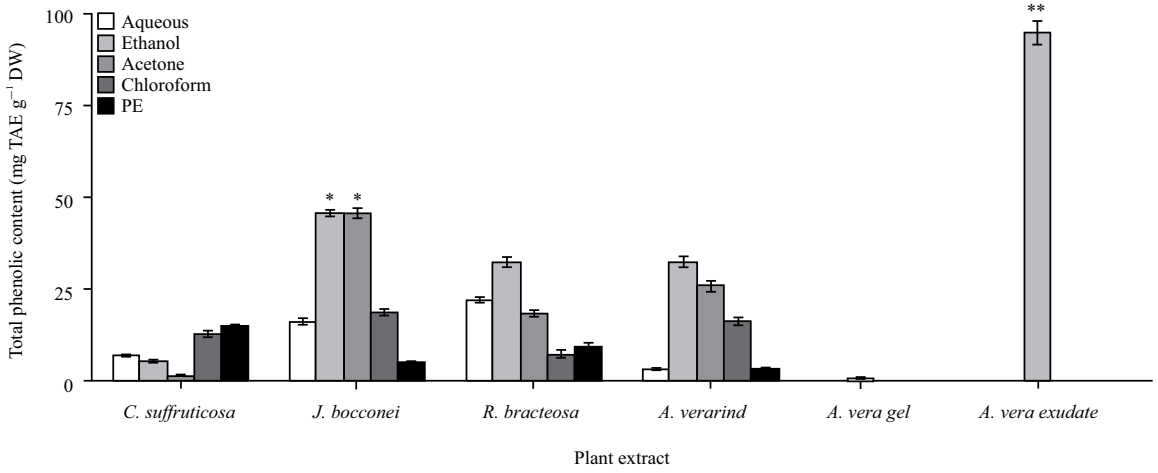

Fig. 1: Total phenolic content per gram DW of extract (mg TAE g^{-1} DW±SEM)
TAE: Tannic acid equivalents, PE: Petroleum ether. *p<0.05, **p<0.01

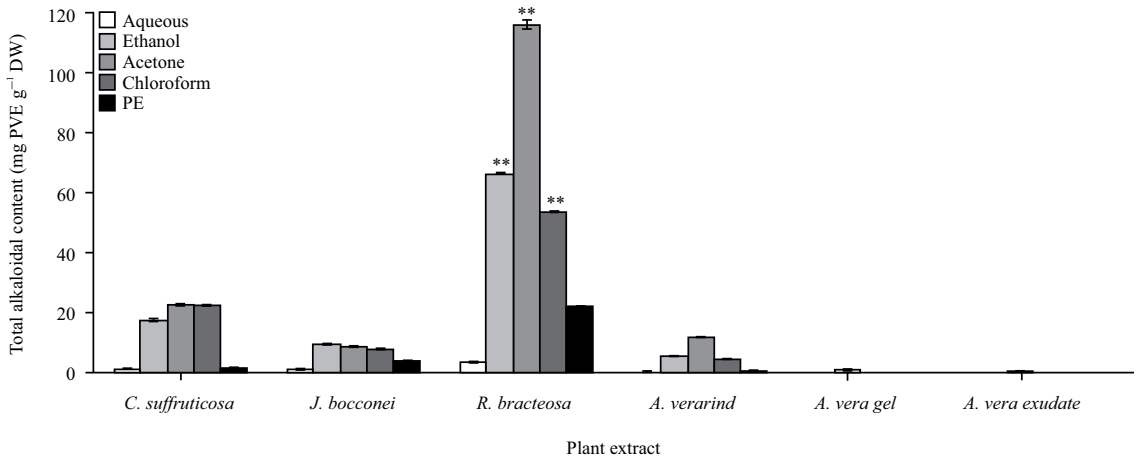

Fig. 2: Total alkaloidal content per gram DW extract (mg PVE g^{-1} DW±SEM)
PVE: Papaverine equivalents, PE: Petroleum ether. **p<0.01

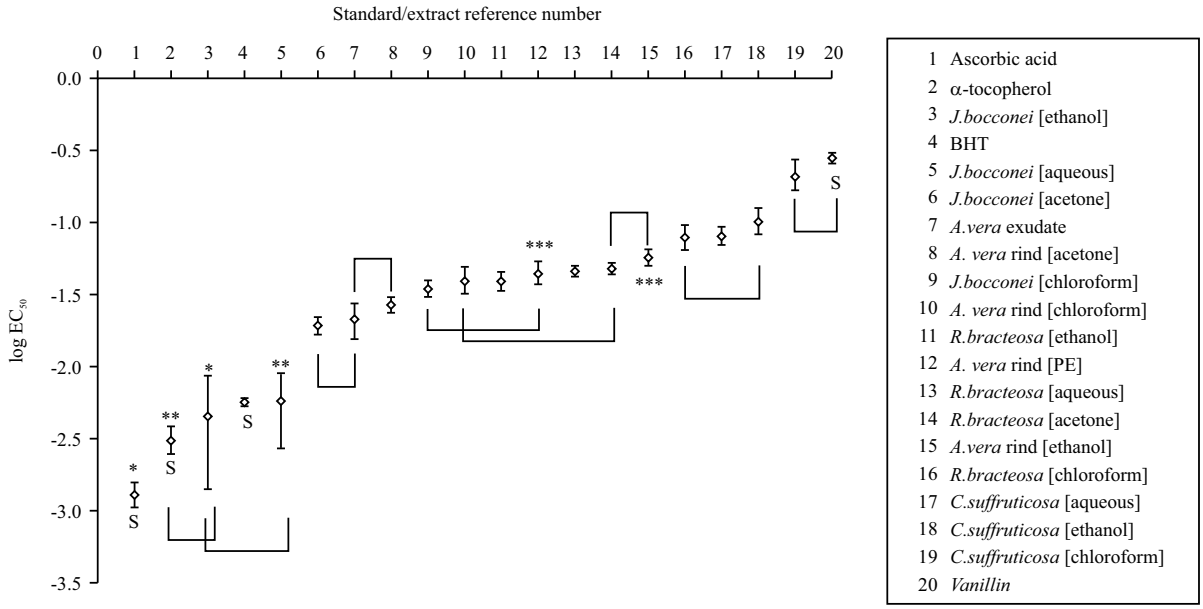

Fig. 3: EC_{50} values (log) for DPPH radical scavenging activity with limits determined by the 95% confidence interval

Except for *A. vera* rind, all the petroleum ether extracts did not reach an EC_{50} value at the concentration of maximum solubility of the extract in the sample vehicle (0.15% w v^{-1}). Besides, at 0.25% w v^{-1}, the EC_{50} was not attained for the acetone extract of *C. suffruticosa*, aloe gel and the aqueous extract of *A. vera* rind. Following analysis of the findings from the phytochemical and antioxidant analyses, further investigations were carried out on aloe exudate; acetone, aqueous and ethanolic extracts.

A decrease in the activity of luciferase was observed after treatment of TNFα-stimulated GloResponse™ NF-κB-RE-luc2P HEK293 cells with the following extracts: *A. vera* exudate and the acetone, aqueous and ethanolic extracts of *J. bocconei*. However, the percentage decrease in luciferase activity following treatment with these extracts never exceeded 20%. Besides, this decrease did not attain significance within the range of concentrations tested. However, treatment with dexamethasone caused a decrease of 29.5% in luminescence from that of untreated cells ($p<0.001$).

Different results were obtained following treatment of GloResponse™ NF-κB-RE-luc2P HEK293 cells with the acetone and ethanolic extracts of *R. bracteosa* (Fig. 4 and 5, respectively). As the concentration of these extracts increased, an increase was observed in the luciferase

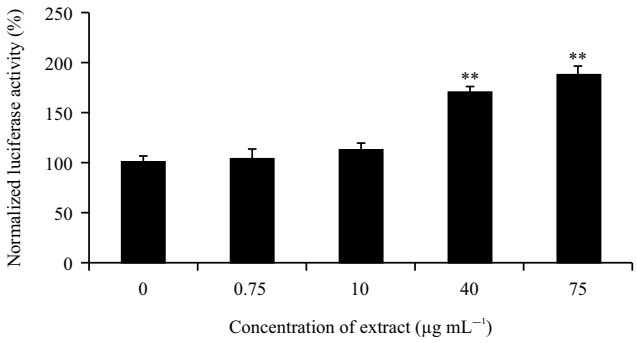

Fig. 4: Effect of the acetone extract of *R. bracteosa* on the luciferase activity of TNFα-stimulated GloResponseTM NF-κB-RE-luc2P HEK293 cells
**p<0.01

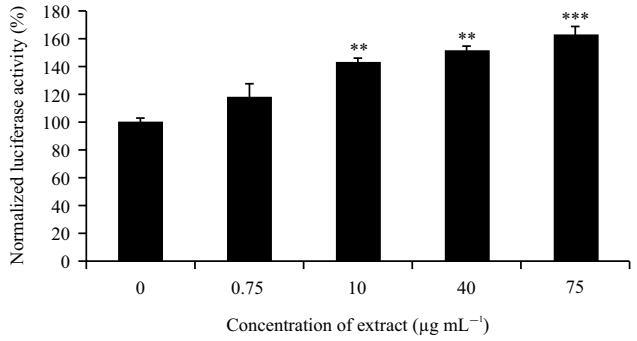

Fig. 5: Effect of the ethanolic extract of *R. bracteosa* on the luciferase activity of TNFα-stimulated GloResponseTM NF-κB-RE-luc2P HEK293 cells
**p<0.01

activity (%). In fact, after treatment with 40 µg mL^{-1} of the acetone and 10 µg mL^{-1} of the ethanolic extracts of *R. bracteosa*, the luciferase activity (%) was noted to increase significantly ($p<0.01$) from 100-170.1% and from 100-142.7%, respectively when compared to extract-untreated cells.

At an extract concentration of 40 µg mL^{-1} which was the highest concentration that was common to all extracts, a significantly positive linear relationship ($p<0.01$) was found between the normalized luciferase activity (%) and the EC_{50} values for the DPPH free radical scavenging activity (%) of the extracts ($r = 0.925$, $n = 6$) (Fig. 6). Besides, a statistically significant positive correlation ($p<0.01$) was found between the normalized luciferase activity (%) and the total alkaloidal content in the extracts ($r = 0.975$, $n = 6$) (Fig. 7).

DISCUSSION

The first part of the study was designed to investigate the phytochemical and antioxidant profiles of twenty-two extracts derived from four Maltese medicinal plants. This study was carried out to determine the pharmacological potential of four Maltese medicinal plants that were used in tradition[30,31]. The data collected from the investigations formed the basis for the selection of six extracts that were subsequently used to investigate their ability to modulate the transcriptional activity of NF-κB.

The findings from the investigation on free radical scavenging activity showed that three or more extracts from each plant exhibited dose-dependent free radical scavenging activity. These findings agree with previous research that had documented similar activity in extracts from plants belonging to the same genera[32-34]. Intracellular levels of ROS modulate the NF-κB transduction pathways in many ways and are capable of modulating the activity of NF-κB[35]. The complexity that characterizes ROS interactions with NF-κB signaling appears to be cell-type specific and owes itself to the several ways in which ROS can simultaneously act on different signaling molecules throughout the transduction pathways. Notably, various structurally unrelated antioxidants have been shown to inhibit NF-κB activation in response to a variety of

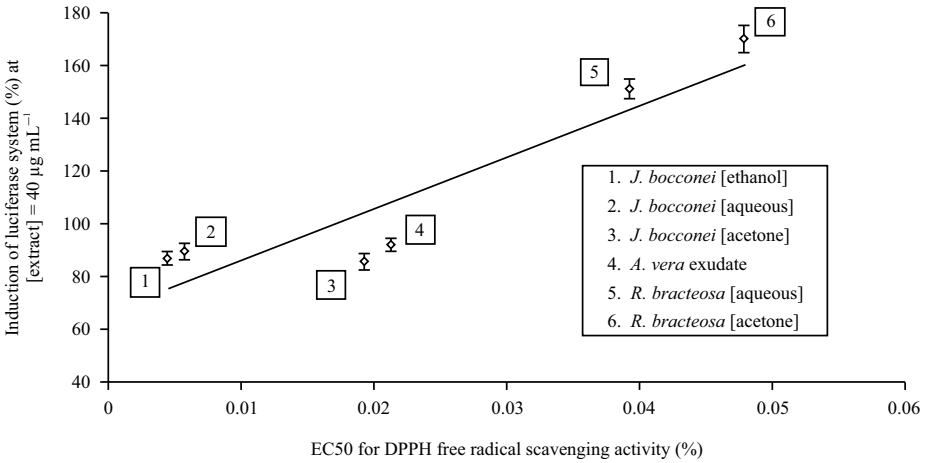

Fig. 6: Statistically significant correlation between the normalized luciferase activity (%) and EC_{50} values for DPPH free radical scavenging activity (%) (n = 6, p<0.01)
Luminescence values were normalized to the luminescence obtained from cells treated with solvent vehicle only (= 100%)

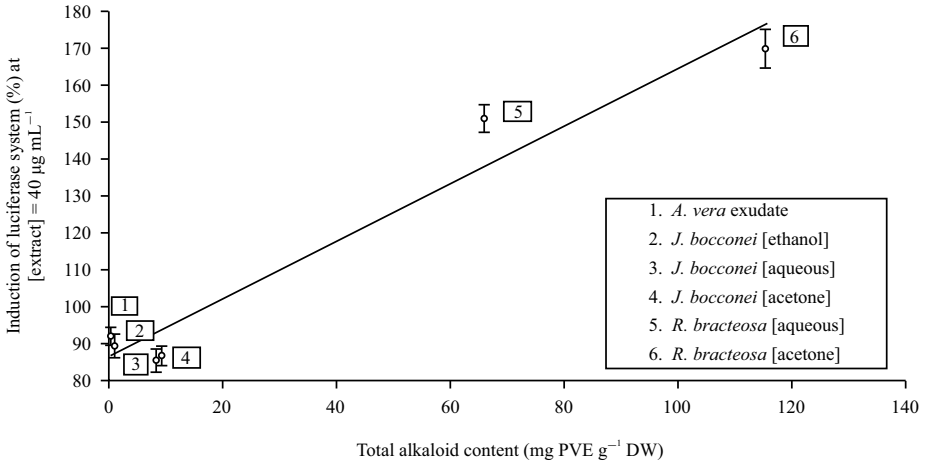

Fig. 7: Statistically significant correlation between normalized luciferase activity (%) and total alkaloid content (mg PVE g^{-1} DW) (n = 6, p<0.01)
Luminescence values were normalized to the luminescence obtained from cells treated with solvent vehicle only (= 100%)

stimuli[36]. In this study, the highest free radical scavenging activity (EC_{50}<0.01% w v^{-1}) was exhibited by the aqueous and ethanolic extracts of *J. bocconei*. Indeed the scavenging activity of these extracts was comparable to that of known potent antioxidant standards. Further research on these extracts was thus warranted to investigate their activity on the transcriptional activity of NF-κB.

In addition to their high free radical scavenging activity, a significantly high phenolic content (p<0.05) was found in the ethanol and acetone extracts of *J. bocconei* when compared to the majority of the other extracts. These findings are in agreement with the documented presence of flavonoids and flavonol glycosides in the aerial parts of members of the genus *Jasonia*[15,37]. Besides, as previously reported by Gutterman and Chauser-Volfson[38], an exceptionally high (p<0.01) phenolic content (94.874 mg TAE g^{-1} DW) was found in aloe exudate. Phenolic compounds tend to exert an inhibitory effect on the transcriptional activity of NF-κB[39]. Besides, it had been documented that various alkaloids modulate the activity of this transcription factor[6,7,11,40]. The highest levels of alkaloids were found in the extracts of *R. bracteosa* which is a known accumulator of a wide range of alkaloids[41]. Because of the phytochemical content and the order of free radical scavenging activity of the extracts (Fig. 3), the most promising candidates for further pharmacological analyses were selected as follows: *A. vera*

exudate, the aqueous, ethanolic and acetone extracts of *J. bocconei* and the ethanol and acetone extracts of *R. bracteosa*.

The findings demonstrated a significant correlation ($p<0.01$) between the EC_{50} for the percentage free radical scavenging actions of the extracts and the transcriptional activity of NF-κB as quantified by luciferase reporter assay (Fig. 6). This agrees with the findings of other studies that reported the activating effect that increased levels of ROS have on NF-κB[42]. The transcriptional activity of NF-κB can be mediated by ROS at various levels of the signal transduction pathway. The mechanisms that are triggered in response to ROS include the phosphorylation and dissociation of IκB from NF-κB, the degradation of IκB, or the activation of regulatory kinases, further upstream along the pathway[43]. However, the results suggest that the free radical scavenging activity was insufficient to cause any significant extract-induced inhibition in NF-κB activity. It had previously been reported that aloe-emodin, an anthraquinone component of the exudate, suppressed the translocation of NF-κB to the nucleus and its binding to DNA in human colon tumor cells[23]. However, other researchers had documented that aloe-emodin did not cause any change in the activation of NF-κB in murine L929 fibrosarcoma cells that were stimulated with interferon-γ and IL-1[22]. In any case, findings related to a single constituent cannot be extrapolated to the biological activity of the crude exudate from the plant.

Notably, treatment of the cells with the acetone and ethanolic extracts of *R. bracteosa* caused an induction in luciferase activity above that of extract-untreated cells. This signifies that constituents in these extracts were inducing upregulation in the transcriptional activity of NF-κB. Although Paur *et al.*[44] had documented similar stimulatory effects of several plant extracts on LPS-induced NF-κB activity in U937 cells, according to our knowledge this is the first study that demonstrates the stimulatory effect of *R. bracteosa* on this transcription factor. The therapeutic significance of this finding is true of pharmacological importance. Although much of the previous research in this field had been invested in efforts targeted to inhibit the activity of this transcription factor, evidence in recent years has demonstrated that stimulation of NF-κB is involved in the induction of apoptotic cell death in a variety of drug-refractory tumor cells[6]. It has been suggested that this could be mediated through the stimulation of the expression of pro-apoptotic genes, such as FAS/APO-1 ligand (FasL) and c-myc. Notably, Ryan *et al.*[4] had shown that p53-induced apoptosis requires NF-κB activation. Besides, they had advanced the hypothesis that inhibition of NF-κB in tumor cells that retain wild type p53 causes a diminished rather than enhanced therapeutic response[4,5]. Thus extracts with the capacity to activate NF-κB should be carefully considered for their potential cytotoxic activity in tumor cells.

Another notable finding in the present study was the statistically strong positive correlation ($p<0.01$) that was found between alkaloidal content and luciferase activity (Fig. 7). The highest luciferase activity was measured after treatment with the ethanolic and acetone extracts of *R. bracteosa* which had the highest content of alkaloids. The activation of NF-κB by plant alkaloids had been documented by other researchers[6,7]. Activation of inhibitory κ kinase (IκK) has been reported to play a critical role in the degradation of IκB and subsequent activation of NF-κB in the regulation of paclitaxel- and vinca alkaloid-induced apoptosis in human tumor cells.

However, the potential anti-tumor potential of alkaloids found in the ethanolic and acetone extracts of *R. bracteosa* remains to be investigated. Besides, further investigation of the effect of the chloroform extract from this plant on the transcriptional activity of NF-κB is warranted, given its high content of alkaloids. It is indeed noteworthy that in a study carried out in Taiwan, cytotoxic activity against human cervix epithelioid carcinoma (HELA) cell line was reported for the alkaloids graveoline and dictamine that were isolated from a methanolic extract of the roots and aerial parts of the related plant *R. graveolens*[45]. In another investigation in the USA on human breast, prostate and colon cancer cells, potent cytotoxic activity was demonstrated by a methanolic extract of the aerial parts of *R. graveolens* through strong inhibition of cell survival and proliferation[46]. These findings substantiate further endeavors undertaken to investigate the chemotherapeutic potential of extracts and alkaloids derived from *R. bracteosa*.

CONCLUSION

This research documents novel findings on the quantitative phytochemical content and free radical scavenging activity of *J. bocconei* and *C. suffruticosa*. Besides, the findings unravel important pharmacological properties of the acetone and ethanol extracts from *R. bracteosa* for which the ability to cause induction in the transcriptional activity of NF-κB has been demonstrated. Notably, a statistically significant positive correlation was attained between the total alkaloidal content in the plants and the activity of NF-κB. Increased transcriptional activity of NF-κB has been linked to cytotoxic activity in a variety of tumor cells.

SIGNIFICANCE STATEMENT

This study discovers the effects of alkaloids as potential inducers of this transcription factor. This study will help the researcher to uncover the critical area of chemotherapy that many researchers were not able to explore. Thus, a new theory on the use of these natural products and possibly analogs of these alkaloidal molecules through chemical synthesis, may be arrived at.

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